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RESEARCH ARTICLE

BIODEGRADATION OF PARACETAMOL BY NATIVE FUNGAL SPECIES INHABITING WASTEWATER OF A PHARMACEUTICAL FACTORY IN SANA'A, YEMEN

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ABSTRACT

Paracetamol has emerged as an important environmental contaminant due to its extensive use. The purpose of this work was to isolate, identify, and characterize fungal species able to degrade paracetamol from pharmaceutical wastewater effluent at Sana'a City, Yemen. The fungi were isolated and purified from wastewater samples using enrichment and selective media. The isolated fungi were identified according to phenotypic characterization. Two species of isolated fungi were able to utilize the paracetamol as the sole of carbon and energy sources. These fungi were designated as F1 and F2 and identified as *Aspergillus niger* and *Fusarium oxysporium*, respectively. Optimum temperature and pH for growth of both species were 25°C and 6.0, respectively. Also, the biodegradation of paracetamol was influenced by glucose concentration. F1 and F2 were able to degrade 35.7% and 26.1% of 1000 and 2000 mg/L, respectively, paracetamol in 60 days. This is the first report on the ability of *Aspergillus niger* and *Fusarium oxysporium* to degrade paracetamol. The reported findings highlight the potential use of the isolated microorganisms for treatment of paracetamol-contaminated wastewater.

Keywords: Biodegradation, fungi, isolation, optimization, Paracetamol.

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INTRODUCTION

Paracetamol or acetaminophen is a common analgesic and an anti-inflammatory widely used as a non-prescription drug that is sold as over-the-counter (OTC) worldwide¹. The paracetamol consumption has increased throughout the world in the past decades in England, it was one of the top three drugs prescribed, while in the USA, it was one of the top 200 prescriptions^{2,3}. In Kuwait, it ranked as the second most-common prescription drug throughout 2008⁴. In Yemen, it was ranked first among the top ten drugs manufactured by local industries, and one of the top ten imported drugs⁵.

Paracetamol is one of the most common detected drug in the aquatic environment; at 0.298 µg/L in drinking water⁶, 6.5 µg/L in ground water⁷, 15.7 µg/L in surface water⁸, 1.367 mg/L in wastewater⁹, and 0.246 mg/L in sewage water¹⁰. Therefore, the potential risk and

effects of paracetamol on the environment and human health have become of concern¹¹.

Previous efforts on the removal of paracetamol from wastewater mainly focused on chemical methods including oxidation processes such as ozonation and H₂O₂/UV oxidation¹² and TiO₂ photocatalysis¹³. The major drawbacks of using these methods are the high operational and energy costs, as well as the generation of secondary pollutants due to the use of excessive chemicals¹⁴. Biodegradation of organic substances is being considered as an environmentally friendly and low-cost option. This process currently receives considerable attention due to its efficiency and ability to degrade different pollutants via the catalytic activity of microbial organisms. In the past decades, biodegradation investigations of paracetamol have largely focused on the use of different bacteria⁴.

The use of fungi as a method of bioremediation provides an alternative to the cleanup of environmental

pollutants. Fungi have recently received significant attention for their bioremediation potential which is attributed to the enzymes they produce¹⁵.

To date, few studies have investigated the potential degradation of paracetamol by fungi. In this study, the isolation, identification, and characterization of new paracetamol degrading fungi from paracetamol-contaminated waste water was performed. The isolated fungi species were further investigated for optimization of growth and paracetamol-degradation parameters. The wastewater was from the Yemen Drug Company for Industrial and Commercial (YEDCO) situated at Sana'a City, Yemen. Since 1982, to date, this company has been generating wastewater containing high concentrations of paracetamol. Therefore, indigenous microbes that were capable of treating various liquid toxic wastes might be present at this site.

MATERIALS AND METHODS

Chemicals and cultivation medium

Chemicals used were paracetamol ultrafine powder (Anqiu Lu'an Pharmaceutical Co. LTD., Chain), and acetonitrile and methanol (Merck, USA). All pharmaceutical standards were of high-purity grade (>99%). The applied basal mineral salts medium (BMSM) consisted of 3.0 g NaNO₃, 0.5 g MgSO₄·7H₂O, 0.5 g KCl, 3.5 g KH₂PO₄, and 0.5 g of Na₂HPO₄ per liter of distilled water. The pH was adjusted between 5.8 to 6.0 and the medium was sterilized at 121°C for 20 min¹⁶.

Paracetamol was added as the sole source of carbon and energy at different concentrations, then the medium was sterilized by filtration (0.22 µm). For preparing solid media, 1.5% w/v agar (Fluka, Bio Chemika, Switzerland) was used.

Sampling, isolation, and purification of fungi

Wastewater samples were collected from the effluent generated by YEDCO factory situated in Sana'a City, Yemen. One milliliter of each sample was transferred to a bottle containing 90 mL of Sabouraud dextrose broth (SDB) media. Each bottle was incubated at 25°C for 5 days. Five milliliters of SDB media were inoculated into BMSM containing 250 mg/L of paracetamol and 0.8 g/L glucose (i.e. enriched medium), and the medium was incubated at 25°C for 14 days^{16,17}.

Subsequently, 1 mL of enriched BMSM was transferred to BMSM agar containing 250 mg/L of paracetamol, and the medium was incubated at 25°C for 15 days. Plates showing growth were subjected to subculturing on Sabouraud dextrose agar (SDA) to obtain pure colonies. Next colonies were transferred several times on BMSM agar containing paracetamol, and finally confirmed to be pure by growth on SDA. Pure colonies were tested for growth on a range of paracetamol concentrations (250–2000 mg/L) in BMSM agar. The isolated fungi that grew well on BMSM agar were tested for growth on higher concentrations of paracetamol in media and characterized further^{16,17}.

Identification of isolated fungi

The isolated fungi were characterized by taxonomic studies and identified as described by Moubasher¹⁸.

Identification of the isolated fungi was performed by macroscopic examination of colony morphology on pure cultures, and by microscopic examination with Lactophenol cotton blue stain¹⁸.

Optimization study

Parameters such as pH, temperature, carbon source, and contact time, were optimized to enable the isolated strains to utilize paracetamol effectively as a carbon and energy source.

Effect of temperature and pH on paracetamol biodegradation

The effects of the temperature and pH on paracetamol biodegradation were evaluated individually for each isolated fungi on BMSM (90 mL) containing 1000 mg/L paracetamol. The isolated fungi were cultured individually on the BMSM and incubated at different temperatures (15, 20, 25, 30, and 35°C) for 30 days. The optimum pH for paracetamol biodegradation was determined using BMSM having different pH levels (4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, and 8) that were incubated at 25°C for 30 days. The pH values were adjusted using 1 N NaOH and 1 N HCl. Samples were withdrawn at regular intervals and centrifuged at 12000 rpm for 15 min. The supernatant was collected in separate clean test tubes and analyzed for measuring the residual paracetamol concentration^{17,19}.

Effect of glucose concentration on the paracetamol biodegradation

The effect of glucose on paracetamol biodegradation was evaluated utilizing different glucose concentrations (0, 1, 2, 3, 4, and 5 mg/L). Each concentration of glucose was added separately to BMSM (90 mL) containing-paracetamol (1000 mg/L), that was inoculated by each fungal species, and that was incubated at 25°C for 60 days. Sampling was done at regular time intervals to determine residual paracetamol levels in medium²⁰.

Effect of incubation period on the different concentration of paracetamol

The effect of incubation period on biodegradation of different concentration of paracetamol was carried out in bottles containing 90 mL of BMSM. Each fungal isolate was cultured individually on BMSM containing different concentrations of paracetamol (250 – 2000 mg/L) and incubated at 25°C at different incubation periods such as 10, 20, 30, 40, 50 and 60 days. After each designated time, the medium was analyzed for residual paracetamol levels.

Chemical analysis methods

Three hundred milligrams of paracetamol working standard was weighed and dissolved in 70 mL of methanol in a 100 mL volumetric flask. 5 mL of solution was transferred to a 100 mL volumetric flask containing 47 mL of methanol and 53 mL of purified water and mixed well. Next, 10 mL of this solution was filtered through a 0.22 µm nylon membrane filter before use in other experiments²¹.

Paracetamol level determination

The residual concentration of paracetamol was determined using HPLC (Perkin Elmer, USA). The mobile phase with a flow rate of 1.0 mL/min consisted of acetonitrile:water (47:53 v/v). The separation was performed at 30°C using a RP-8 column (5 µm,

4.6×250 mm). The injection volume was 20 µm, retention time was 5 min, and detection wavelength was fixed at 275 nm²¹.

RESULTS

Identification and characterization of isolates

The YEDCO factory wastewater was chosen as the source for isolating microorganisms in this project. Two fungal species capable of degrading paracetamol at different concentration were isolated from the wastewater samples and designated as F1 and F2, which were later identified as *Aspergillus niger* and *Fusarium oxysporium*, respectively, according to morphological characterization.

Figure 1 shows color change on the BMSM agar containing paracetamol by F1 and F2. Figure 2 shows the change in color of BMSM broth after several days of incubation indicating the formation of paracetamol degradation products.

Optimization study

Effect of temperature and pH on paracetamol biodegradation

The effects of temperature and pH on the biodegradation rate of paracetamol (1000 mg/L) were determined after 30 days. The temperature range selected for the biodegradation of paracetamol was 15 to 35°C. Maximum biodegradation of paracetamol was 30.6% and 51.4% observed at 25°C by F1 and F2, respectively, after 30 days. A decrease in paracetamol degradation was reported at temperatures greater than 30°C or less than 20°C for both F1 and F2 (Figure 3).

The effect of pH on paracetamol degradation was investigated at various initial pH values (4.0–8.0). The optimum pH for paracetamol degradation was 6.0 for both species. F1 and F2 degraded 30.6% and 51.4% of 1000 mg/L paracetamol within 30 days, respectively. However, degradation efficiency was relatively high across a pH range of 5.5–6.5. The degradation rate decreased at a pH greater than 7.0 or smaller than 5.0 (Figure 4).

Effects of incubation period on biodegradation of different concentrations of paracetamol

The paracetamol biodegradation by F1 and F2 was evaluated at different concentrations of paracetamol at different incubation periods. F1 degraded 45.06% and 35.7% of 750 mg/L and 1000 mg/L, respectively, of paracetamol in 60 days. Also, 100% and 52.4% of paracetamol were degraded at an initial concentration of 250 and 500 mg/L, respectively, at 50 days (Figure 5). F2 degraded 41% and 26.1% of paracetamol in 60 days at an initial concentration of 1500 mg/L and 2000 mg/L, respectively. At 750 and 1000 mg/L, 86.93% and 68.9% of paracetamol were degraded in 60 days, respectively, (Figure 6).

Effect of glucose on paracetamol biodegradation

The effects of different concentrations of glucose on paracetamol biodegradation were investigated for F1 and F2 on BMSM containing-paracetamol (1000 mg/L) at 25°C for 60 days. Paracetamol biodegradation increased with increasing glucose concentration for both fungal species. At 5 mg/L of glucose, 100% and 91.3% of 1000 mg/L paracetamol were degraded by F1

and F2 species, respectively, after 60 days (Figure 7 and Figure 8).

DISCUSSION

Microorganisms play a significant role in biological decomposition of hazardous compounds present in the environment. Several fungi are known to degrade persistent pollutants. The results of the present study demonstrated that a site contaminated with pharmaceutical wastewater effluent containing paracetamol is rich with variety microorganisms able to utilize paracetamol as a sole source of carbon and energy. Two fungal species signed as F1 and F2 were isolated from the waste water effluent contaminated site and were identified as *Aspergillus niger* and *Fusarium oxysporium*, respectively. Similarly, Mendonça *et al.*,²² isolated *F. flocciferum* from an industrial effluent containing phenol. Also, it was isolated *Penicillium* sp. from a paracetamol-contaminated site¹⁶.

Fungi are applied in degradation of persistent organic contaminants due to their general oxidative enzymatic system, that includes ligninolytic extracellular enzymes as laccase and peroxidases, as well as intracellular enzymes as the cytochrome P450 system^{23,24}. Kamaraj *et al.*²⁵ isolated *Aspergillus* sp. from a tannery effluent as a bisphenol A degrader. Also, it was isolated *Penicillium* sp. CHY-2 from Antarctic soil that is able to use some aliphatic and aromatic hydrocarbons as a sole source of carbon and energy²⁶. Raaman *et al.*²⁷ isolated an *Aspergillus* sp. from the polythene polluted sites around Chennai.

Temperature plays a vital role in biodegradation and gives an understanding to degradation pathways for paracetamol. Activities of the enzymes produced by the fungi are influenced directly by temperature. In this study, the optimum temperature for paracetamol biodegradation was 25°C for both isolated fungi. Increasing or decreasing temperature from the optimum 25°C levels decreased rate of paracetamol biodegradation. An important abiotic factor affecting microbial metabolism is pH. The biodegradation of paracetamol was affected by the initial pH of the culture medium. The optimum pH for paracetamol degradation was 6.0 for both fungal species.

This result is consistent with that by Marinho *et al.*²⁸ who reported that the optimum pH for atrazine degradation by *A. niger* was 5. Also, Govarthanam *et al.*²⁶ recorded that decane degradation by *Penicillium* sp. CHY-2 was high (at 81%) at pH 6.0 and 25°C after 28 days. Another study by Yemendzhiev *et al.*²⁹ reported that the maximum degradation of a phenol and cresol mixture by *Aspergillus awamori* was at pH of 5.5 and 25°C. In contrast, to these results, Kamaraj *et al.*²⁵ found that the optimum pH for bisphenol A removal by *Aspergillus* sp. was at pH 9.0. The carbon source of the growth medium is an important factor for the growth and metabolism of a microorganism. Selection of an ideal and economical carbon source accelerates the growth of the fungus as well as the biodegradation process³⁰. Glucose is a rapidly metabolized substrate by fungi. Therefore, fungi appear

to have a higher affinity to glucose than other carbon sources²⁸.

In this study, paracetamol biodegradation was enhanced by increasing glucose concentration in the culture medium. Similarly, it was reported that the benzo(a) pyrene degradation by *Fusariumsolani* was approximately 9% and 50% in presence of 1 g/L and 10 g/L of glucose, respectively³¹. It was evaluated the effect of glucose on the removal of methyl parathion by *A. niger* AN400, and found that the presence of 0.5 mg/L glucose increased the rate of removal of methyl parathion²⁸. A study by Govarthanam *et al.*²⁶ indicated that the addition of 5 g/L of glucose enhanced decane degradation by about 1.8-fold at 20°C, and reported that glucose is the most suitable carbon source for the growth of *Penicillium* sp. CHY-2. Also, Marinho *et al.*³² reported degradation of atrazine (30 mg/L) by *A. niger* AN400 in presence of glucose³².

Fungi are used to degrade a wide variety of materials and compounds, a process known as mycodegradation³³. In this work, the ability of the isolated fungal species was evaluated at different concentrations of paracetamol with different incubation periods. F1 was able to degrade 100% of 500 mg/L of paracetamol at 50 days and 35.7% of 1000 mg/L in 60 days. Also, F2 degraded 86.93% and 26.10% of 750 and 2000 mg/L, respectively, of paracetamol within 60 days. A similar study by Hart and Orr¹⁶ first reported the degradation of paracetamol by an isolated fungal species identified as a *Penicillium* sp. This species possessed the ability to utilize paracetamol as the sole carbon source for growth. Also, Cruz-Morató *et al.*³⁴ experimented with the treatment of paracetamol using a *Trametes versicolor* in a batch fluidized bed bioreactor containing a concentration of paracetamol between 109.3 µg/L–114.4 µg/L. Paracetamol was completely removed by *T. versicolor* after 8 days.

Similarly, Mendonça *et al.*²² reported that *F. flocciferum* was able to reduce the phenolic concentration from 200 mg/L to below detection limits in 24 h. In addition, it was reported that the *Fusarium* sp. E033 was able to degrade 65–70% of benzo(a) pyrene (100 mg/L) within 30 days of incubation at 32°C³⁵. Also, it was reported that the 77% of 20 ppm of bisphenol A was removed by *Aspergillus* sp.²⁵ Another study by Raaman *et al.*²⁷ found that *A. japonicus* and *A. niger* degraded 12% and 8% of polyethylene, respectively, in 30 days. Also, a study by Hasan³⁶ observed that 93% of the kerosene concentration was degraded by *A. niger* after 28 days of incubation. A study by Govarthanam *et al.*²⁶ reported that *Penicillium* sp. CHY-2 was able to degrade 34.0% of decane and 25.0% of butylbenzene (500 mg/L each) at 20°C after 28 days.

Few studies up to now have been reported about the using of fungi enzymes to degrade the paracetamol. It was combined two fungal enzymes, laccase (*Trametes versicolor*) and tyrosinase (mushroom), into a cross-linked enzyme aggregate which was used to transform paracetamol from wastewater samples. It was found that more than 80% of paracetamol present in municipal wastewater and hospital waste water, were transformed by the cross-linked enzymes³⁷.

CONCLUSION

Pharmaceutical wastewater effluent was considered the most potential source to isolate microorganisms able to utilize paracetamol as sole carbon and nitrogen sources. Two fungal species were isolated from the wastewater and identified as *A. niger* and *F. oxysporium*. The isolated were able to survive in the presence of paracetamol concentrations up to 1500 mg/L. The fungal species identified herein, are reported for the first time as paracetamol degraders. Temperature and pH influenced the rate of paracetamol degradation. Therefore, this work has provided a useful guideline in estimating potential paracetamol degraders isolated from the environment. An advanced study of the metabolic pathways involved in paracetamol degradation may increase our understanding on the mechanisms involved in the biodegradation process.

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CONFLICT OF INTEREST

No conflict of interest associated with this work.

REFERENCES

- Martindale W. The complete drug reference, cough suppressants, expectorants, mucolytics and nasal decongestants, 36th ed. The pharmaceutical Press, London, England. 2009; 1082.
- Sebastine IM, Wakeman RJ. Consumption and environmental hazards of pharmaceutical substances in the UK. *Process Saf Environ Prot.* 2003; 81:229-235.
- Zhang X, Wu F, Wu XW, Chen P, Deng N. Photodegradation of acetaminophen in TiO₂ suspended solution. *J Hazard Mater.* 2008; 157:300-307.
- Alajmi HM. Effect of physical, chemical and biological treatment on the removal of five pharmaceuticals from domestic wastewater in laboratory-scale reactors and a full-scale plant. Ph.D. dissertation, University of Newcastle Upon Tyne, England, UK. 2014; 50-87.
- Edrees WH, Abdullah QY, AL-Kaf A, Naji KM. A review on comparative study between the physicochemical and biological processes for paracetamol degradation. *Univ J Pharm Res.* 2017; 2:9-13.
- Kleywegt S, Pileggi V, Yang P, Hao C, et al. Pharmaceuticals, hormones and bisphenol A in untreated source and finished drinking water in Ontario, Canada: Occurrence and treatment efficiency. *Sci Total Environ.* 2011; 409:1481-1488.
- Zimmerman MJ. Occurrence of organic wastewater contaminants, pharmaceuticals, and personal care products in selected water supplies. Cape Cod, Massachusetts, June 2004: US, Geological Survey Open-File Report 1206, 2005; 1-16.
- Lin YA, Tsai T. Occurrence of pharmaceuticals in Taiwan's surface waters: Impact of waste streams from hospitals and pharmaceutical production facilities. *Sci Total Environ.* 2009; 407: 3793-3802.
- Thomas KV, Dye C, Schlabach M, Langford K. Source to sink tracking of selected human pharmaceuticals from two Oslo city hospitals and a wastewater treatment works. *J Environ Monit.* 2007; 9:1410-1418.
- Gomez MJ, Bueno MJM, Lacorte S, Fernandez-Alba AR, Aguera A. Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast. *Chemosphere.* 2007; 66: 993-1002.

11. Wu S, Zhang L, Chen J. Paracetamol in the environment and its degradation by microorganisms. *Appl Microbiol Biotechnol.* 2012; 96:875-884.
12. Andreozzi R, Caprio V, Marotta R, Vogna D. Paracetamol oxidation from aqueous solutions by means of ozonation and H₂O₂/UV system. *Water Res.* 2003; 37: 993-1004.
13. Yang L, Yu LE, Ray MB. Degradation of paracetamol in aqueous solutions by TiO₂ photocatalysis. *Water Res.* 2008; 42:3480-3488.
14. Trovo AG, Melo SS, Nogueira RP. Photodegradation of the pharmaceuticals amoxicillin, bezafibrate and paracetamol by the photo-Fenton process: Application to sewage treatment plant effluent. *J Photochem Photobiol A.* 2008; 198: 215-220.
15. Husaini A, Roslan HA, Hii KSY, Ang CH. Biodegradation of aliphatic hydrocarbon by indigenous fungi isolated from used motor oil contaminated sites. *World J Micro Biotech.* 2008; 24(12): 2789-2797.
16. Hart A, Orr DL. The degradation of paracetamol (4-hydroxyacetanilide) and other substituted acetanilides by a *Penicillium* species. *A Van Leeuw.* 1975; 41:239-247.
17. Ahmed S, Javed AM, Tanvir S, Hameed A. Isolation and characterization of a *Pseudomonas* strain that degrades 4-acetamidophenol and 4-aminophenol. *Biodegradation.* 2001; 12: 303-309.
18. Moubasher AH. Soil fungi of Qatar and Other Arab Countries. The scientific and applied research centre, University of Qatar, Doha, Qatar. 1993.
19. Khan AS, Hamayun M, Ahmed S. Degradation of 4-aminophenol by newly isolated *Pseudomonas* sp. strain ST-4. *Enzyme Microb Tech.* 2006; 38:10-13.
20. Khan SA, Hamayun M, Khan AL, Ahmad B, Ahmed S, Lee J. Influence of pH, temperature and glucose on biodegradation of 4-aminophenol by a novel bacterial strain, *Pseudomonas* sp. ST-4. *African J Biotech.* 2008; 8(16): 3827-3831.
21. United States Pharmacopoeia (USP), 30-NF/25. Monograph: Paracetamol (acetaminophen). The United States Pharmacopoeial Convention, Rockville, USA. 2005; 30(5):172-174.
22. Mendonça E, Martins A, Anselmo AM. 2004. Biodegradation of natural phenolic compounds as single and mixed substrates by *Fusarium flocciferum*. *Electronic J Biotech.* 2004; 7(1): 30-37.
23. Asgher M, Bhatti HN, Ashraf M, Legge RL. Recent developments in biodegradation of industrial pollutants by white rot fungi and their enzyme system. *Biodegradation.* 2008; 19:771-783.
24. Kues U. Fungal enzymes for environmental management. *Curr Opin Biotech.* 2015; 33:268-278.
25. Kamaraj M, Manjudevi M, Sivaraj R. Degradation of bisphenol a by *Aspergillus* sp. isolated from tannery industry effluent. *Int J of Pharm Life Sci.* 2012; 3(4): 1585-1589.
26. Govarthanan M, Fuzisawa S, Hosogai T, Chang Y-C. Biodegradation of aliphatic and aromatic hydrocarbons using the filamentous fungus *Penicillium* sp. CHY-2 and characterization of its manganese peroxidase activity. *RSC Adv.* 2017; 7: 20716-20723.
27. Raaman N, Rajitha N, Jayshree A, Jegadeesh R. Biodegradation of plastic by *Aspergillus* spp. isolated from polythene polluted sites around Chennai. *J Acad Indus Res.* 2012; 1(6): 313-316.
28. Marinho G, Rodrigues K, Araujo R, Pinheiro ZB, Silva GM. Glucose effect on degradation kinetics of methyl parathion by filamentous fungi species *Aspergillus niger* AN400. *Eng Sanit Ambient.* 2011; 16(3): 225-230.
29. Yemendzhiev H, Gerginova M, Zlateva P, Stoilova I, Krastanov A, Alexieva Z. Phenol and cresol mixture degradation by *Aspergillus wamori* strain: Biochemical and kinetic substrate interactions. *Proceedings of ECO pole.* 2008; 2(1):153-159.
30. Launen L, Pinto L, Moore M. Optimization of pyrene oxidation by fungi using response surface analysis. *Appl Microbiol Biotechnol.* 1999; 51: 510-515.
31. Verdin A, Sahraoui AL-H, Newsam R, Robinson G, Durand R. Polycyclic aromatic hydrocarbons storage by *Fusarium solani* in intracellular lipid vesicles. *Environmental Pollution.* 2005; 133:283-291.
32. Marinho G, Barbosa BA, Rodrigues K, Aquino M, Pereira L. Potential of the filamentous fungus *Aspergillus niger* AN 400 to degrade Atrazine in wastewaters. *Biocatalysis and Agricultural Biotechnology.* 2017; 9:162-167.
33. Singh H. *Mycoremediation: Fungal Bioremediation.* John Wiley and Sons, Inc., Hoboken, New Jersey, Canada. 2006; 38.
34. Cruz-Morató C, Lucas, D, Llorca M, Gorga M, Rodríguez-Mozaz S, Barceló D, Marco-Urrea E, Sarrà M, Vicent T. Hospital wastewater treatment by fungal bioreactor: Removal efficiency for pharmaceuticals and endocrine disruptor chemicals. *Sci Total Environ.* 2014; 493:365-376.
35. Chulalaksananukul S, Gadd GM, Sangvanich P, Sihanonth P, Piapukiew J, Vangnai AS. Biodegradation of benzo(a) pyrene by a newly isolated *Fusarium* sp. *Federation of European Microbiological Societies (FEMS Microbiol Lett).* 2006; 262: 99-106.
36. Hasan IF. Biodegradation of Kerosene by *Aspergillus niger* and *Rhizopus stolonifer*. *J App Envir Micro.* 2014; 2(1): 31-36.
37. Ba S, Haroune L, Cruz-Morató C, Jacquet C, Touahar IE, Bellenger J, Legault YC, Jones JP, Cabana H. Synthesis and characterization of combined cross-linked laccase and tyrosinase aggregates transforming acetaminophen as a model phenolic compound in wastewaters. *Sci Total Environ.* 2014; 487:748-755.

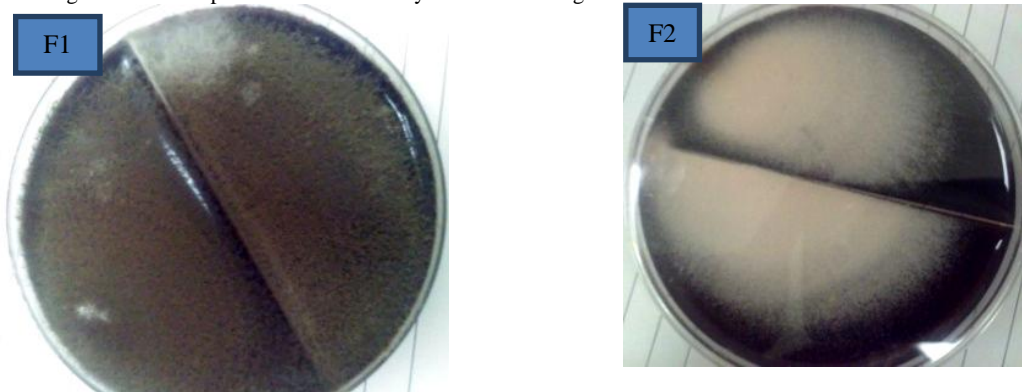


Figure 1: F1 and F2 species growth on BSM agar containing paracetamol

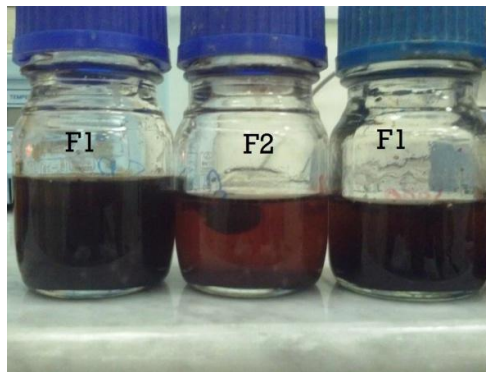


Figure 2: Growth of F1 and F2 in BSM broth.

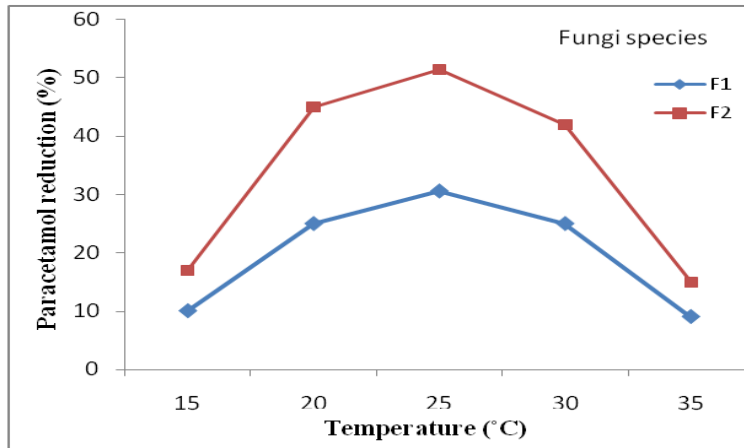


Figure 3: Effect of different temperatures on paracetamol biodegradation

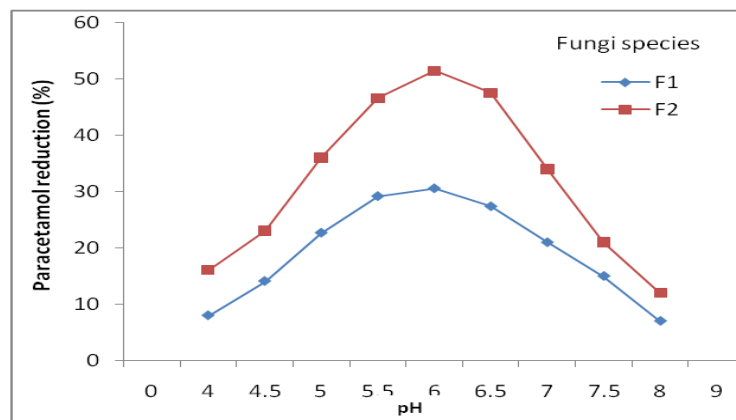


Figure 4: Effect of pH on paracetamol biodegradation.

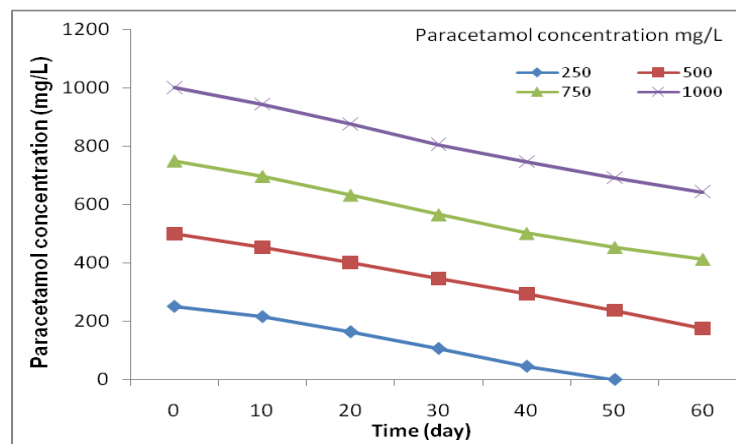


Figure 5: Paracetamol degradation curve by F1.

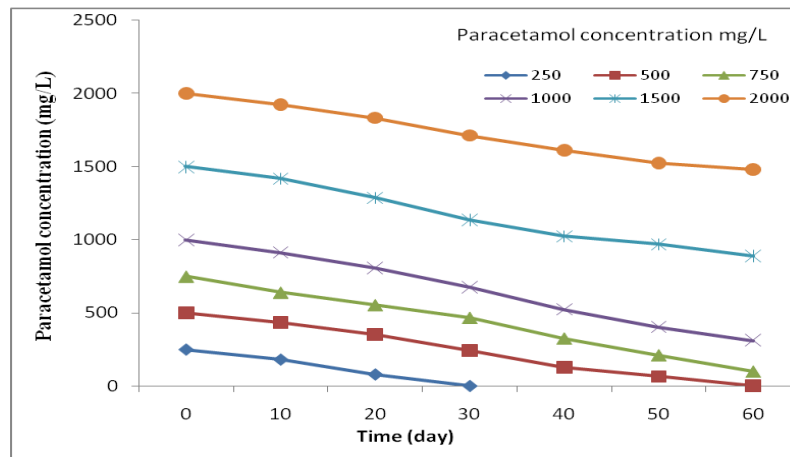


Figure 6: Paracetamol degradation curve by F2.

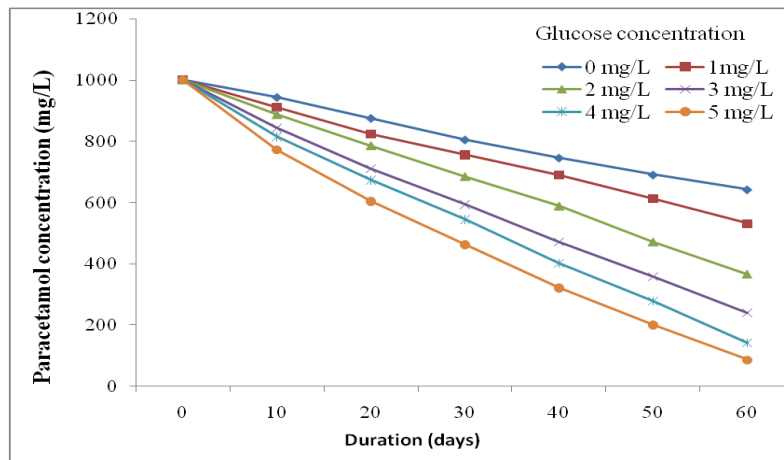


Figure 7: Effect of glucose supplementation to medium on paracetamol biodegradation by F1

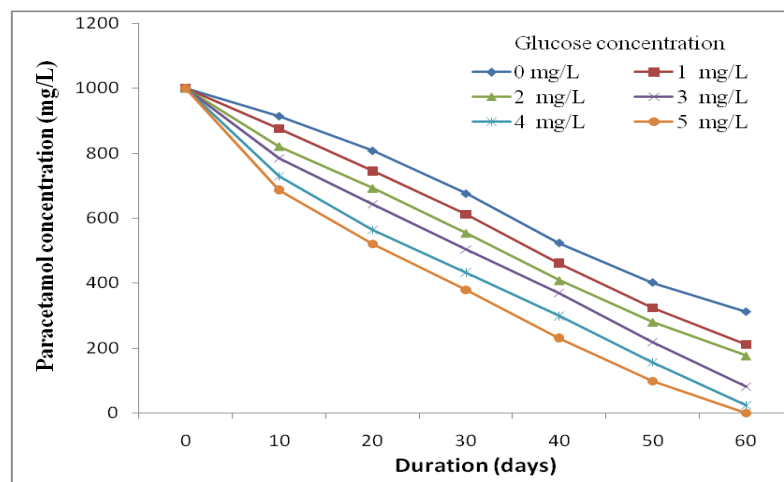


Figure 8: Effect of glucose supplementation to medium on paracetamol biodegradation by F2