British Biotechnology Journal
4(3): 317-324, 2014

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Identification of Bacterial Population of Activated Sludge Process and Their Potentials in Pharmaceutical Effluent Treatment

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FM and RJE designed the study. Authors FR and GN, performed the statistical analysis. Authors FM and RJE wrote the protocol. Authors NF, NJM and RJE wrote the first draft of the manuscript. Authors RJE and NJM managed the analyses of the study. Authors NF and RJE managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

Received 16th November 2013
Accepted 1st February 2014
Published 19th February 2014

ABSTRACT

Aims/objectives: The cognition about microbial population of activated sludge and their treatment potential will be very useful for industrial wastewater treatment plant operation.
Methodology: In this study microbial population of activated sludge process that was used for pharmaceutical wastewater has been investigated. Sampling was done from return sludge line and after serial dilution 1500 plates were studied. Methods for separating the bacteria from wastewater was pour plate method. All bacterial samples were purified using nutrient Agar and Macconkey Agar culture. Bacteria were separated from return sludge line and classified into 3 groups after biochemical tests and

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morphological analysis, These include positive bacteria of Bacillus genus, Pseudomonas aeruginosa and Flavobacterium.
Results: The biodegradability study on pharmaceutical effluent using identified cultures in laboratory scale showed that Bacillus spp. are the most efficient bacteria for organic matter degradation.
Conclusion: Results of this study showed that providing a microbial bank of these spp. can be useful for resistant operation of activated sludge.

Keywords: Pharmaceutical wastewater; microbial population; activated sludge; COD.

1. INTRODUCTION

Nowadays one of the most important environmental contaminants is pharmaceuticals. It is expected that their worldwide production will increase, and substantial amounts of pharmaceuticals can reach the environment, either through direct discharge into the water resources or due to the inefficient removal in wastewater treatment plants [1]. There is little similarity between characteristics of wastewater from different pharmaceutical factories. Commonly these effluents contain little biological oxygen demand (BOD) and it can be negligible but contain higher chemical Oxygen Demand (COD) [2].

The Biological processes are commonly used for industrial wastewater because these methods are economical and environmental sound. Most industrial wastewaters contain multi-component mixture of various types of organics [3,4]. These organics can be broadly classified as, readily degradable or bio-refractory. Certain pharmaceuticals wastewater contains a large amount of bio-refractory and toxic compound [4]. Therefore biological treatment of pharmaceuticals wastewater will be very difficult and Conventional biological treatment plants are usually inappropriate for the treatment of pharmaceutical effluent [5].

When treating industrial wastewaters, particularly for removal of toxic or refractory organics, it is necessary acclimate the biomass to wastewater and conservation of acclimated biomass specially, bacteria are considered as an important action [5].

Identification of bacterial population as the most important organisms for wastewater treatment can be very useful for stable operation of wastewater treatment plant. Aerobic and anaerobic bacteria are used for pharmaceutical wastewater treatment [6]. Many studies on pharmaceutical wastewater treatment plant shows that the microbial population is vary from one plant to another and wastewater characteristics and environmental factors affecting the microbial population [5,6]. It has been found in some cases the acclimated biomass to possess a genetic memory [7]. This is of great importance for those industries with intermittent production of specific products such as pharmaceutical industry.

The main aims of this study were: to identify the bacteria that are involved in pharmaceutical wastewater treatment in an activated sludge process, to determine their efficiencies for COD removal and determine the most efficient bacteria and provide a bacterial bank for seeding to the activated sludge process in emergencies condition such as hydraulic, organic and toxic shocks.

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals were purchased from Merck and Aldrich companies in analytical grade.

2.2 Experimental Methods

2.2.1 Microbial culture

The first step in screening was providing pure culture or mixed population with the highest removal efficiency. Sampling was done for 1 month from return sludge line and sent to laboratory in a safe and standard manner. The volume of each sample was 4 liter. The samples were diluted by serial dilution method to 7 dilutions (10^1, $10^{2,}$, ……10^7). All diluted sample were cultured and incubated at 37ºC by spread plate and pour plate method with about 1500 plate evaluated. To make pure culture, each plate was sub-cultured in 3 tubes containing nutrient agar.

2.2.2 Determination of treatment potential of bacteria

Potential of activated sludge for pharmaceutical wastewater treatment was first determined using 48 hr. In this method 4 reactors with volume of 10 liter were selected and aerated by a blower (Fig.1). The mixture of sterilized pharmaceutical wastewater and activated sludge in the reactors 1, 2, 3, and 4 were as shown in Fig 1. For evaluation of treatment potential of different bacterial cultures (3 identified cultures) for pharmaceutical wastewater, 5 days aeration method was used. This method was conducted same as 48 hr method except that 5 day aeration and pure culture instead of activated sludge were used.

2.3 Analytical Methods

All tests and analytical methods conducted according to the standard methods for the examination of water and wastewater [6].

3. RESULTS AND DISCUSSION

Table 1 shows the specification of bacteria in dilution of 10^5. It can be seen so many colonies are growth on both of culture nature with different specification.

Table 2 shows the classification of identified bacteria as morphological and biochemical characteristics. Table 3 show the results of biochemical tests.

The results of biochemical tests revealed that, it can be concluded that the MS 3-1 is *Pseudomonas aeruginosa*, the P2M10^{-4}2 is *Flavobacterium* spp, and the P1N10^{-3}3 is *Bacillus* spp. with their distribution in the activated sludge as *Pseudomonas aeruginosa* (50%), *Flavobacterium* spp. (13.5%), and bacillus spp. (1.19%) [8]. These microbes can be maintained and exploited for efficient maintenance and operation of wastewater treatment plant [9,10]. These results conformed to earlier studies [11,12]. Moreover, results of this study indicated the antibacterial effects of engineered nanomaterials implications for wastewater treatment plantsThe *Pseudomonas aeruginosa* spp Specific baced D.G.G.E (denaturing gradient gel electrophoresis) [13].

Table 1. Specification of bacteria in dilution of 10^5

No	specification culture nature		Number of colonies	Apparent specification of colonies	Number of bacteria	Number of gr^+	staining
1	N.A	First plate	150	Dispersed milky colonies	5×10^7	33	gr^+ gr^-
		Second plate	125	Milky dispersed colonies some smooth and some hard	12.5×10^7	6	gr^+ gr^-
2	Mac .A	First plate	17	Silver and red dispersed colonies and aerobic	11.7×10^7	-	gr^-
		First plate	19	Dispersed pink colonies and aerobic	11.9×10^7	0	gr^-

gr^- : Gram negative; gr^+ :Gram positive; N.A: Nutrient agar; Mac. A: macconkey agar

Table 2. Classification of bacteria from morphological and biochemical points of view

No	Oxidative-fermentative test	Cotalas test	Oxides test	Form and characteristics of bacteria	Specification of colonies on nutrient agar and Mac cankey agar	specification vcxzVCZcgf
1	+ +	+ +	+ +	gr^- Coccus and diplo coccus gr^-	Blue colonies on nutrient agar	MS-3-1* M4-2P
2	+ + + +	+ + + +	+ + + +	gr^-	Fine to medium brown Medium brown Clear chocolate brown Geometrical cream colonies	P2m10^{-4} 1 P1m10^{-4} 1 P1m10^{-3} 2 P2m10^{-3} 2
3	+ + + +	+ + + +	+ + + +	gr^-	Big brown mucous chocolate Brown-chocolate Brown to violate Small brown mucous	P2m10^{-4} 3 P2m10^{-4} 2* P1m10^{-4} 2 P1N10^{-3} 1
4	+	+	-	gr^+	Yellow colonies 1-2 ml on nutrient agar	P1N10^{-3} 3

+ : Positive; - : negative; gr^- : Gram negative; gr^+ :Gram positive;
Gr^- Coccus: Coccus Gram negative diplo coccus gr^- : diplo coccus Gram negative

Table 3. Biochemical tests for identification of MS 3-1, P2M10^{-4}2 and P1N10^{-3}3 bacteria

Type of Bacteria	Morphological	Negative	Positive
MS 3-1	Gr $^-$ double coccus	glucose, lactose, sucrose, SH_2 ,indole , $Co_2^+H_2$, VP , Of with oil	MR, Citrate, oxides, Of, catalase, Malonate, Motility
P2M10^{-4}2	Bacillus gr $^-$	Lactose ,sucrose, SH_2 indole , $Co_2^+H_2$,VP, Motility	MR , glocuse,Citrate, oxidase ,Of, catalase, Malonate, Of with oil
P1N10^{-3}3	bacillus gr $^+$ spore forming	SH_2, indole, $Co_2^+H_2$,VP Motility, Malonate , oxides ,Of	MR, catalase, citrate Of with oil

Methods were developed and important in many WWTPS (Wastewater treatment plants). (Please check grammar of this sentence) Results of this study indicated that pharmaceuticals reduced diversity of microbial community of activated sludge [15]. The results of biochemical tests have confirmed that the MS 3-1 is *Pseudomonas aeruginosa*, the P2M10^{-4}2 is *Flavobacterium* spp, and the P1N10^{-3}3 is Bacillus spp. As a result of Lapra et.al, research, percents of identified bacteria in activated sludge was *Pseudomonas aeruginosa* (50%), *Flavobacterium* spp. (13.5%), and *Bacillus* spp. (1.19%) [8]. According to the study of Rani et al., the most microbial diversity in activated sludge was related to bacillus and pseudomonas. These microorganisms help us to reach efficient maintenance and operation in wastewater treatment plant [9]. Also this results with study [11,12] Moreover Results of this study indicated the antibacterial effects of engineered nanomaterials implications for wastewater treatment Plants [13,14]. Results of this study indicated that pharmaceuticals reduced diversity of microbial community of activated sludge [15]. (Are these sentences replicated? Please check it).

Fig. 1 shows the decreasing of COD after 3, 6, 12, 24 and 48 hr were reported as treatment efficiencies.

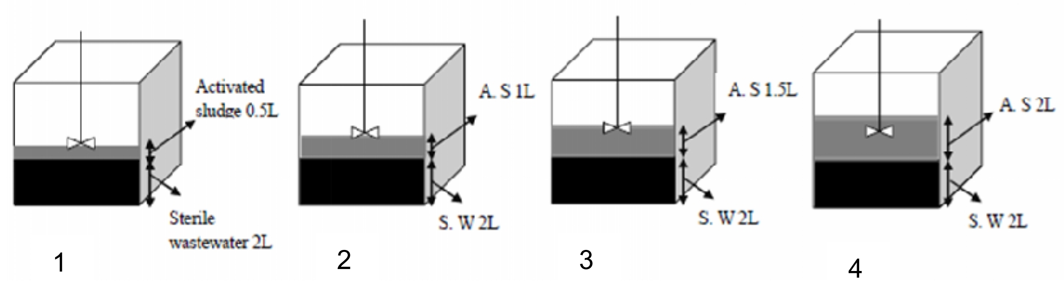

Fig. 1. Wastewater treatment steps in lab scale using activated sludge

Fig. 2 Shows the potential of activated sludge for treating pharmaceutical waste water in different concentration. The more concentration of activated sludge is seeded, the more efficiency of COD reduction is achievable.

It can be seen that after 24 hr the COD reduction is constant and increasing the aeration time have no effect on COD decreasing, therefore it can be resulted that optimum detention time of aeration tank is 24 hr [8,16].

These results are consistent with results of LaPara et al. [8]. That showed the COD removal was about 62% at 30ºC and the extent of soluble COD removal declined as temperature increased by an average of 60 mg/L per ºC [5]. (Please check grammar of this sentence)

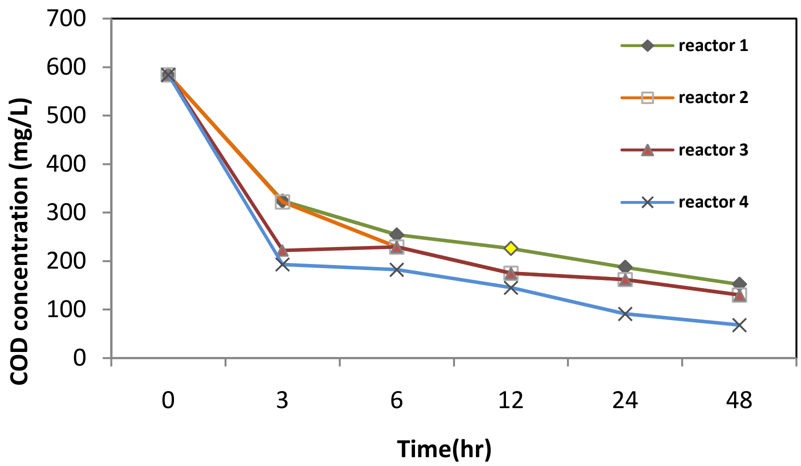

Fig. 2. The potential of activated sludge for removal of COD

Fig. 3 shows the experimental set up for evaluation of treatment potential of identified bacteria for pharmaceutical wastewater.

- As shown in Fig. 4, *Bacillus* spp. is the most efficient bacteria for pharmaceutical wastewater treatment. However no significant differences among the efficiencies of three bacteria spp (PV=0.99). It is not consistent with results of Madukasi [17] that reports *Rhodobacter spheroidies* Z08 has proven to be effective in ameliorating hazardous pollutants found in pharmaceutical wastewater with over 80% COD reduction [5]. It can be seen when pure culture are used for wastewater treatment the optimum aeration time increased (2 days) in comparison to mixed population as activated sludge. The reason is related to role of other organisms in wastewater treatment such as fungi, protozoa, etc.

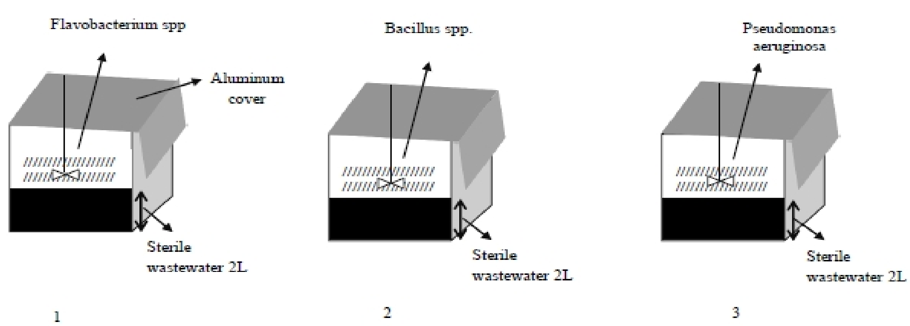

Fig. 3. Wastewater treatment steps in lab scale using isolated bacteria

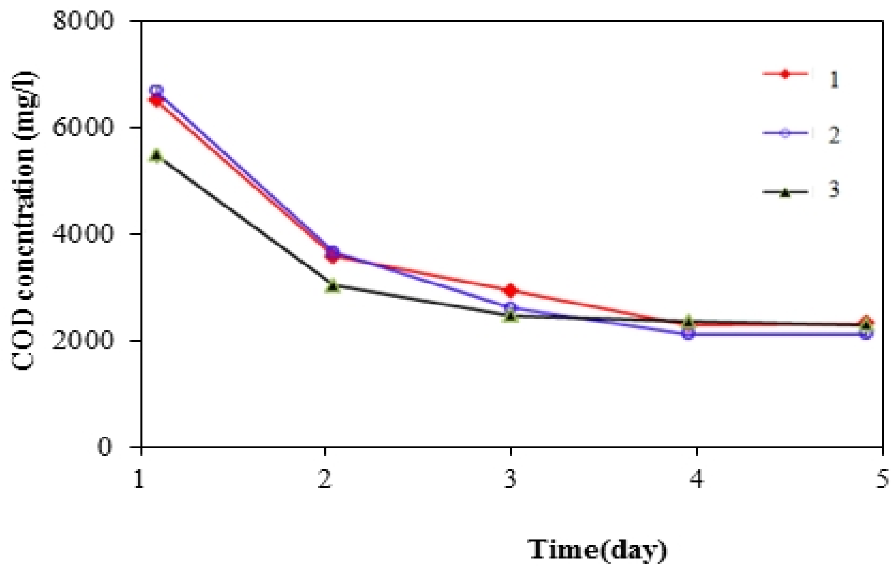

Fig. 4. The COD removal efficiencies of each of identified bacteria in pharmaceutical wastewater treatment.

4. CONCLUSION

Results from this study indicate that the pharmaceutical wastewater can be treated by activated sludge within 24 hr aeration time.

Three species of bacteria are identified as following

1- *Flavobacterium* spp.
2- *Bacillus* spp.
3- *Pseudomonas aeruginosa*

Bacillus spp. were the most efficient bacteria for pharmaceutical wastewater treatment, and providing a microbial bank of this spp can be useful for resistant operation of activated sludge and prevention of effects of toxic, organic and hydraulic shocks.

ACKNOWLEDGEMENT

I would like to express my special thanks to the Guilan university of medical science - Faculty of Health officials for Financial and moral support of this project.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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