Efficiency of fungi suspension spores as Biocoagulants for suspended solid sedimentation in wastewater

*Khalid Falih Hassan** *and Saba Hussein Obeid*

ABSTRACT: Fungi *Aspergillus, Penicillium* and *Trichoderma* suspension spores were used as Biocoagulants to sediment suspended solid in wastewater for its negative impact in wastewater treatment units. Suspension spores 173-182× 10^2 spore\ml, 152-163× 10^4 spore\ml and 122-133× 10^6 spore\ml were used to reduce turbidity in concentration of 167 NTU and pH 7.8 in batch system and 15, 30, 45 and 60 min period treatment. Results showed that fungi suspension spores by 122-133× 10^6 spore\ml have high ability to reduce turbidity concentration and approach of alum in 84%. This study is the first record for using fungi suspension spores as Biocoagulants .

Keywords: Biocoagulants , Fungi , spore suspension

INTRODUCTION :

Coagulation is aggregation of small particles suspended in the water with each other due to different electrical charge and forming the large particles that can be removed easily during the Sedimentation or Filtration process[1]. As it has been the adoption on physical and chemical processes for sedimentation of the particles suspended in the water column with small volumes. It has been found a very small particles, it do not be removed by using physical and chemical methods in coagulation processes , which is less than 10 microns in size and which cause clogging of filters and form a favorable environment for the growth of microorganisms , thereby causing negative effects on public health and economic damage [2] .

As well as, the coagulants of chemical industrial polymer were used in water treatments, such as derivatives of polyacrylamide which have effects toxic , carcinogenic and dangerous on human health, as well as the aluminum element which is the main component of poly aluminum chloride causes Alzheimer's disease and a serious neurological diseases[3]. Biocoagulation , which is efficient technologically and environment-friendly precipitates suspended particles from the waste water and has a high-capacity to decrease the concentrations of some pollutants . In their study [4] *Mycobacterium phlei* were used as biocoagulant , where this microorganism produces the extracellular superficial polymeric materials in certain conditions , it has a high-capacity on linking the solid particles with each other and cause their sedimentation. The study of [5] proved the use of microorganisms as biocoagulant , which linked with the particles suspended leading to change the shape and size of these particles and caused their sedimentation . The study of [6] showed that *Saccharomyces cerevisiae* and *Yarrowia lipolytica* have the

Khalid Falih Hassan: BSc. Biology Scientific Research in Ministry of Science & Technology\ Environment &Water Researches Dep.\ Iraq

*E- mail alrubbay@gmail.com

ability to reduce the concentrations of each of the metals of zinc sulfide and lead sulfide.

In the study of [7] , it was proved that the seeds of plants *Moringa peregrina fiori* and *Moringa oliferalam* have wide applications for removing turbidity of pollutants water because they contain active compounds . The current study aims to prove the efficiency of using spore suspension for fungi *Aspergillus, Penicillium* and *Trichoderma* as biocoagulant to precipitate the particles suspended in wastewater and compare them with alum.

MATERIAL AND METHODS :

1- Collection of wastewater samples: Samples of waste water were collected from the preliminary stage sedimentation of Russtamyiah Treatment Plant (wastewater treatment station in Baghdad) by using sterile glass bottles for isolating of fungi . Other samples were collected from waste water by plastic bottles(5 Liter) and transferred to the laboratory , then they were sterilized by autoclave at 121^0 C and 1.5 bar for using in experimentations .

2- Alum solution : Alum solution was prepared at concentration of 10 ppm.

3- Isolation , cultivation and diagnosis of fungi : *Aspergillus niger* , *Trichoderma harizanum* and *Penicillium requeferts* were isolated from wastewater and they were cultivated according to [8]. Malt extract agar(MEA) was prepared and chloramphenicol antibiotic was added (prepared previously by dissolving 250 gm from antibiotic to 250 ml of D.W) . One milliliter of sample was put in sterile glass plates (9 cm) and then MEA was added in plates that contain the samples . Plates were incubated in 25^o C for 48 hour. Microscopic slides were prepared after growth of the fungal colonies for diagnosis of fungal species by depending on the taxonomy key [9].

4- Suspension spores: Ten ml of distilled water were added in each plates which are containing the pure isolates of *A. niger* , *T. harizanum* and *P. requeferts* . Thereafter, five ml of distilled water were took from each plate and transferred to sterile glass bottles containing 95 ml of sterile saline solution for per fungus then shook completely . Spores were calculated and used the dilution method to obtain the required number of spores [10].

5- Calculations :

A- The spores of fungi were enumerated in 1 ml of suspension by Hemocytometer (counting chamber) [11] according to the equation :

The number of spores (spore /ml) = the number of spore in 4 squares × 4× 10

B- The rate of reduction =(Initial concentration – Final concentration)x 100

6- The experiment: One liter of each samples of the sterile wastewater were placed in glass cylinder (capacity of one liter). Alum was added at concentration of 10 ppm for one sample in volume 10 ml \ Liter which is the ideal dose for sedimentation according to [3] to compare it with the spore suspension. The spore suspension of *A. niger* , *T. harizanum* and *P. requeferts* ($173x\,10^2$ -$133x\,10^6$ spore\ml) were separately added to sample of wastewater . The concentrations of turbidity were measured before and after the treatment for periods 15, 30,45 and 60 min by using Turbidity meter[12] . Sample of wastewater was used without any addition as the control.

RESULTS AND DISSCUTION :

Table (1) shows the reduction of turbidity concentrations from waste water by using alum solution and suspension spores of *P. requeferts* , *A. niger* and *T. harizanum* for periods 15,30,45 and 60 min with the number of spores ranged 173-182×10^2 spore\ml . The concentration of turbidity in water waste before treatment was 176 NTU . The concentration of turbidity decreased to 120.95, 66.7 and 25 NTU after 15, 30, 45 and 60 min respectively, after treatment by alum at concentration of 10 ppm with volume 10 ml / L to precipitate the particles suspended. While , the concentration of turbidity was decreased to 144, 129.5, 110.6 and 85.7 NTU after treatment by suspension spores of *T. harizanum* (177 × 10^2 spore / ml) in the same periods of treatment . The concentration of turbidity was 145.3, 132.2, 112.4 and 80 NTU for periods of treatment 15,30, 45 and 60 min respectively, when suspension spores of *A. niger* was used with the number of spores (173 × 10^2 spore / ml) . Whilst the concentration of turbidity declined to 140.6, 125.3, 104.8 and 78.5 NTU in the same treatment periods during the treatment by suspension spores of *P. requeferts* (182 × 10^2 spore / ml).

Table (1) shows the reduction of turbidity concentrations from waste water(turbidity = 176 NTU) by using alum solution and suspension spores of *P. requeferts* , *A. niger* and *T. harizanum* with the number of spores 173-182× 10^2 spore\ml

Coagulant	The concentration of coagulant	The concentration of turbidity after treatment NTU			
		15 min	30 min	45 min	60 min
Control		174	169.2	158	142.6
Alum	10 ppm	120	95	66.7	25
Suspension spores for *Trichoderma harizanum*	177×10^2 spore\ ml	144	129.5	110.6	85.7
Suspension spores for *Aspergillus niger*	173×10^2 spore\ ml	145.3	132.2	112.4	80
Suspension spores for *Penicillium requeferts*	182×10^2 spore\ ml	140.6	125.3	104.8	78.5

Table (2) shows the reduction of turbidity concentrations from waste water by using alum solution and the suspension spores of *P. requeferts* , *A. niger* and *T. harizanum* for periods 15,30,45 and 60 min with the number of spores ranged 152-169 x 10^4 spore\ml . The concentration of turbidity decreased to 150.4, 100.8, 88.8 and 40.5 NTU after the treatment by suspension spores of *T. harizanum* (155 x 10^4 spore\ml) in the same periods of treatment respectively . The concentration of turbidity became 152, 98.7, 85 and 41.3 NTU by using suspension spore of *A. niger* (163 × 10^4 spore / ml) for periods of treatment 15.30, 45 and 60 min respectively . Whilst the concentration of turbidity decreased to 151.8, 102, 85.7 and 42.4 NTU in the same treatment periods by treating suspension spores of *P. requeferts* (152 × 10^4 spore / ml).

Table (2) shows the reduction of turbidity concentrations from waste water(turbidity = 176 NTU) by using alum solution and suspension spores of *P. requeferts* , *A. niger* and *T. harizanum* with the number of spores 152-163× 10^4 spore\ml

Coagulant	The concentration of coagulant	The concentration of turbidity after treatment NTU			
		15 min	30 min	45 min	60 min
Control		174	169.2	158	142.6
Alum	10 ppm	120	95	66.7	25
Suspension spores for *Trichoderma harizanum*	155×10^4 spore\ ml	150.4	100.8	88.8	40.5
Suspension spores for *Aspergillus niger*	163×10^4 spore\ ml	152	98.7	85	41.3
Suspension spores for *Penicillium requeferts*	152×10^4 spore\ ml	151.8	102	85.7	42.4

Table (3) shows the reduction of turbidity concentrations from waste water by using alum solution and suspension spores of *P. requeferts* , *A. niger* and *T. harizanum* for periods 15,30,45 and 60 min with the number of spores ranged 122-133 x 10^6 spore\ml . The concentration of turbidity decreased to 185, 182.3, 60.2 and 32.4 NTU after treatment by suspension spores of *T. harizanum* (125 x 10^6 spore\ml) in the same periods of treatment .The concentration of turbidity became 192, 187, 58 and 28.5NTU by using suspension spores of *A. niger* (133 × 10^6 spore / ml) for periods of treatment 15.30, 45 and 60 min respectively . Whilst, the concentration of turbidity decreased to 190.6, 180, 52.6 and 27.6 NTU in the same treatment periods by treating suspension spores of *P. requeferts* (122 × 10^6 spore / ml).

Table (3) shows the reduction of turbidity concentrations from waste water(turbidity = 176 NTU) by using alum solution and suspension spore of *P. requeferts* , *A. niger* and *T. harizanum* with the number of spores 122-133 × 10^6 spore\ml

Coagulant	The concentration of coagulant	The concentration of turbidity after treatment NTU			
		15 min	30 min	45 min	60 min
Control		174	169.2	158	142.6
Alum	10 ppm	120	95	66.7	25
Suspension spores for *Trichoderma harizanum*	125×10^6 spore\ ml	185	182.3	60.2	32.4
Suspension spores for *Aspergillus niger*	133×10^6 spore\ ml	192	187	58	28.5
Suspension spores for *Penicillium requeferts*	122×10^6 spore\ ml	190.6	180	52.6	27.6

The results proved the ability of suspension spores for fungi selected on reduction the concentration of turbidity with high efficiency compared with control and alum . The reduction rate of turbidity concentration for suspension spore of fungi was approached from the reduction rate by using alum .The results were concurred with[13] as they proved in their study the ability of *Saccharomyces cerevisiae* and *Yarrowia lipolytica* to sedimentate of particles of sulphide less than 10 micron (particles galena and sphalerite) with high efficiency and these microorganisms used as bio coagulants. As well as the results were compatible with [14] in their study to reduce the concentration of pyrite and chalcopyrite as small particles with high efficiency by using bacteria *Acidithiobacillus ferrooxidans*.

Figures (1 ,2, 3) show direct proportionality between the ratio of reduction the concentration of turbidity from wastewater after the 60 min and increase the number of

spores which were used as bio coagulants , the ratio of reduction was 51, 54 and 55% when suspension spore was used with the number of spore 177 × 10^2, 173 × 10^2 and 182 × 10^2 Spore / ml respectively the reduction ratio increased to 77.77 and 76% with rising the number of spores to 155 × 410, 163 × 410 and 152 × 410 Spore / ml, respectively. when the number of spores in spore suspension were increased to 125 × 10^6, 133 × 10^6 and 122 × 10^6 Spore / ml , the ratio of reducing concentration turbidity was increased to 81.84 and 84% respectively.

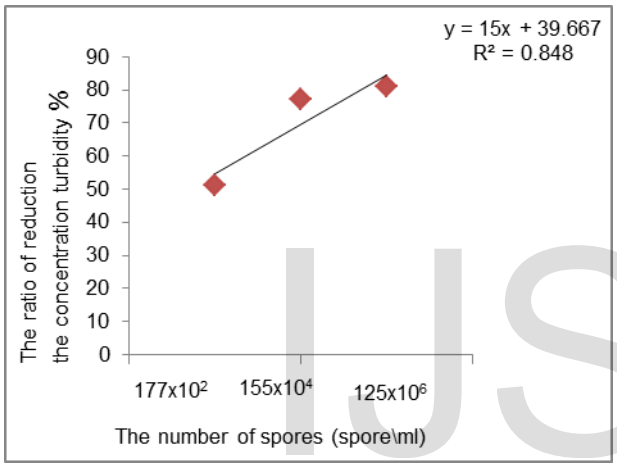

Figure (1) The relationship between the ratio of reduction the concentration of turbidity from wastewater and the number of suspension spore for fungus *Trichoderma harizanum*

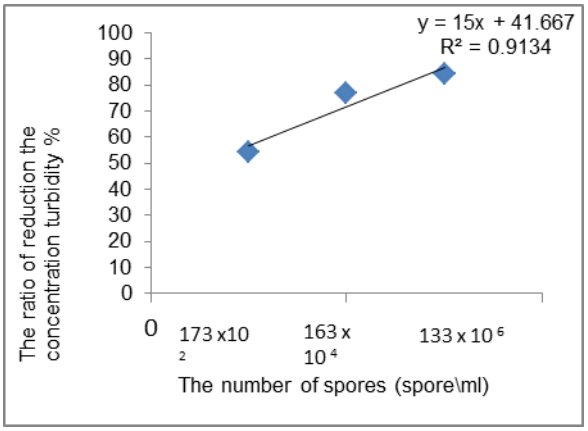

Figure (2) The relationship between the ratio of reduction the concentration of turbidity from wastewater and the number of suspension spore for fungus *Aspergillus niger*

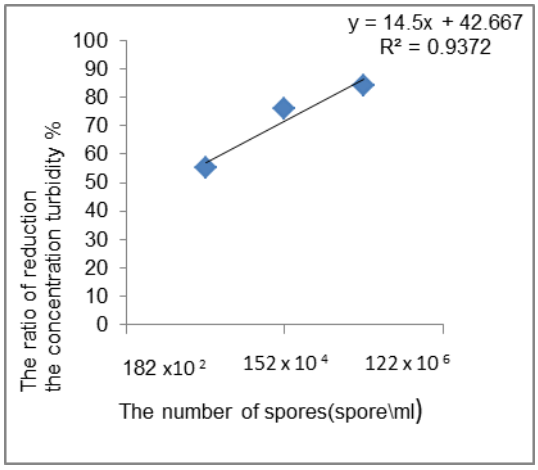

Figure (3) The relationship between the ratio of reduction the concentration of turbidity from wastewater and the number of spore suspension for fungus *Penicillium requeferts*

Therefore, the current results have proved increasing the number of spores lead to decline in the concentration of turbidity because the cell wall of fungal spore consist of two layers of fatty acid , layer of amino acid ,polysaccharide and terminal group of ions hydroxyl and carboxyl which are negatively charge ; thereby they give negative charge to spores [15] . These electrical properties are giving the ability for spores to link with compounds that have opposite charge including heavy metal , cyclic compounds , free ions and other compounds within waste water [16] . The external appearance of coarse for fungal spores (Figure 4) gives the large surface area to link small particles and compounds which give fungal spore the ability to adsorb small particles [17].

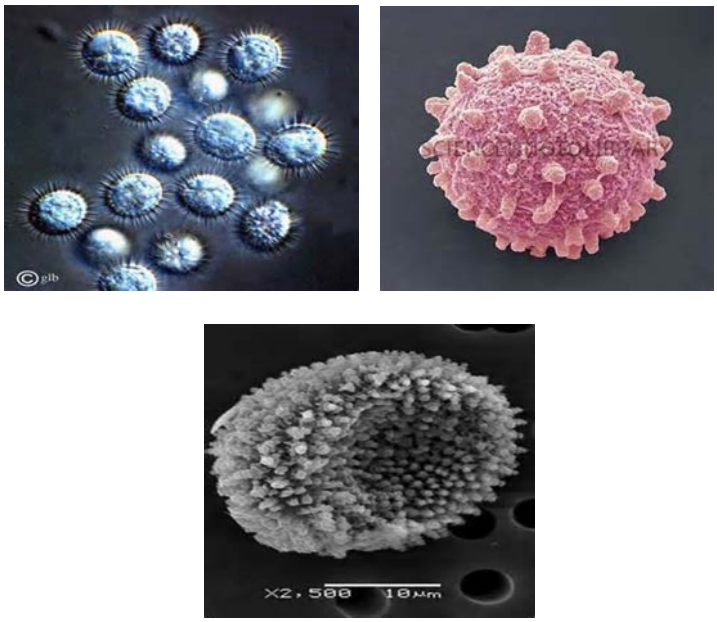

Figure (4) The external appearance coarse for fungal spore[18]

CONCLUSION :

It was observed that suspension spore of fungi were able to reduce the concentration of turbidity from wastewater . Results showed that fungi suspension spores by 122-133× 10^6 spore\ml have high ability to reduce turbidity concentration and approach of alum in 84%.

ACKNOWLEDGMENT:

We thanks to Noor Nihad Baqer & Dr.Inaam Noori Ali for supporting and gratefully thankful ministry of science and technology \ Environment &Water Researches Dep.\ Iraq

References :

1- Davis , M. L. (2010) Water and Waste Water Engineering Design Principles and Practice Copyright by The McGraw-Hill Companies, Inc. Pp 225-246.

2- Lin, d. Q.; Brixius, P. J.; Hubbuch, J. J. ; Tommes, J. and Kulam, z. (2003). Biomass/adsorbent electrostatic interactions in expanded bed adsorption: A Zeta potential study [JJ]. Biotechnol. Bioeng.Vol.(2). pp: 149-157.

3- World Health Organization. (2004).Water Treatment and Pathogen Control: Process Efficiency in Achieving Safe Drinking Water. Edited by Mark W LeChevallier and Kwok-Keung Au. Published by IWA Publishing, London, UK.

4- Long, S. and Wagner, F.(1987) .Structure and properties of biosurfactants. Biosurfactants and Biotechnology. Vol. (2) .pp: 21-46.

5- Capes, C. E; McIllhinney, A. E.; Sirianni, A. F. and Puddington, I. E. (1973). Bacterial oxidation in upgrading pyritic coals. Canadian Mineral and Metal Bulletin. vol. (66). pp: 88-91.

6- Kuyumcu , H.Z ; Pinka,J and Bielig, T. (2008) A novel process for sorting fine-sized sulphide minerals by biocoagulation Trans. Nonferrous Met. Soc. China 18 :1473-1479.

7- Ndabigenesere, A. and Subba Narasiah, K. (1998). Use of Moringa Oleifera seeds as a primary coagulant in wastewater treatment. Enviromental Technology.Vol.(19). pp: 789-800.

8- Romero, M.C.; Hammer, E.; Hanschke, R.; Arambarri, A.M. and Schauer, F. (2005). Biotransformation of biphenyl by filamentous fungus *Talaromyces helicus*. World Journal of Microbiology and Biotechnology, vol. 21, no. 1, p. 101-106.

9- Webster, J. (1980), Introduction to fungi.2nd ed. Cambridge University Press, New York.Pp:669.

10- Moor, E. and landecker, A. (1972). Fundamentals of Fungi. Prentice-Hall India, private limited, New Delhi, pp. 265-277.

11- Busscher, H. J.; Weerkamp, A. H.; van der Mei, H. C.; van Pelt, A. W.; de Jong, H. P. and Arends, J. (2000). Measurement of the surface free energy of bacterial cell surfaces and its relevance for adhesion. Applied and Environmental Microbiology. vol. (48). pp. 980-983.

12- APHA (1998). Standard method for the examination of water and wastewater, 16th ed. American public Health Association, American water works Association and water pollution control federal, Washington, D.C.

13- Halit, Z. K.; Tina, B.; Annamaria, V. and Hanumantha, K. R. (2009). Biocoagulation and its Application Potentials for Mineral Bioprocessing. The Open Mineral Processing Journal. Vol. 2: 1-11.

14- Vilinska, A. and Hanumantha, K. R. (2009). Surface thermodynamics and extended DLVO theory of *Acidithiobacillus ferrooxidans* cells adhesion on pyrite and chalcopyrite. The Open Colloid Science Journal. Vol. 2:1-14.

15- Deacon, J. W. (1989), Introduction to Modern Mycology. In Basic Microbiology. Volume 7. Editor J.F. Wilkinson, Blackwell Scientific Publications, Oxford. Pp: 68-78.

16- Eva-Maria, D. (2007). Fundamentals of Biological Wastewater Treatment. Udo Wiesmann, In Su

Choi, WILEY-VCH Verlag Gmb H & Co. KGaA, Weinheim. Pp: 360-382.

17- Ingold, C. T. (1984). The Biology of Fungi. Fifth Edition, Hutchinson& Co. (Publishers) Ltd., London. Pp: 478-490.

18- Moss, S. T. (2005). The Biology of Fungi. Cambridge University Press, Sydney. Second edition. Pp: 722-735.