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Influence of a Low-frequency Magnetic Field on the Growth of Microorganisms in Activated Sludge

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ABSTRACT

Activated sludge treatment systems for wastewater treatment are increasingly used worldwide, and their efficiencies in the removal of organic matter are high. However these depend on the growth and development of the microorganisms that compose it, therefore generating a stimulus that allows to increase the microbial growth to have an impact on the removal. One of the methods that are currently being researched to stimulate this growth is the use of low-frequency magnetic fields. Therefore, this article presents the response of different microorganisms contained in an activated sludge at magnetic field densities of 5, 10 and 20 mT in periods of time of 30, 60 and 120 minutes, finding that for magnetic fields of 10 mT for 60 minutes and 20 mT for 30 minutes there is an increase in the growth rate close to 68% in bacteria, while for fungi the greatest increase was found in the magnetic field of 5 mT during all exposure times close to 50%. On the other hand, there was also an inhibitory effect for bacteria and fungi in magnetic fields of 10 and 20 mT for different times.

INTRODUCTION

The effect of magnetic fields on microorganisms has been studied worldwide for different purposes. One of these is the possibility of being used to improve wastewater treatment systems that use microorganisms, mainly activated sludge systems (Niu et al. 2014, Tomska & Wolny 2008). There are a variety of microorganisms that intervene in this process, such as bacteria, fungi, protozoa and algae. Bacteria are the most important microorganisms in the process of activated sludge, since they produce the decomposition of the organic matter of the effluent, and the different biochemical activities of the bacteria allow them to metabolize a large part of the organic compounds that are present in the wastewater (Seviour & Blackall 2012).

Applications on the impact of bacterial growth and its effects on the removal of contaminants have been carried out on treatment systems, pilot plants and experimental cases in the laboratory. For example, Lebkowska et al. (2013) conducted a study of activated sludge in a magnetic field of 7 mT, finding an increase of 20% in the elimination of formaldehyde. In addition, the magnetic field also had a positive effect both on the efficiency of chemical oxygen demand, in addition to increasing the rate of nitrification and the elimination of ammonia. As for the bacteria and the growth of the biomass, it was actively stimulated, giving a growth of the mud biomass together with the number of bacteria and the microfauna present, generating a protective effect on the metabolic activity of activated sludge. Niu et al. (2014) presented some effects of different magnetic field intensities varying between 10 to 50 mT on activated sludge activity, which has been compared to low-temperature conditions (5°C) in a biological reactor, where the dehydrogenase activity was intensified at 40 mT. However, from 20 to 40 mT it could stimulate the microorganisms to produce unsaturated fatty acids to adapt to the low temperature. On the other hand, the Gram-negative bacteria with better adaptability to the cold were enriched to a great extent, which guarantees the cold resistance of a low temperature reactor, concluding that the optimum intensity that strengthens the activity and cold resistance of the microorganisms present in the activated sludge in the biological treatment of wastewater at low temperature was for this case 30 mT. Tomska & Wolny (2008) applied a magnetic field in the process of the activated sludge, where a reduction of nitrogen compounds was found, such that the nitrification process is more effective under the influence of a magnetic field of density of 40 mT, due to a stimulation of the microorganisms responsible for these processes. Finally, Zaidi et al. (2014) found that a magnetic field has the potential to minimize the proliferation of filamentous microorganisms, thus reducing the appearance of swollen or bulking sludge. Filipiè et al. (2012) presented the effect of a low-frequency magnetic field on the bacteria *Escherichia coli* and *Pseudomonas putida* on their biological activities in the treatment of wastewater. A decrease in the growth of both microorganisms was obtained at exposures of 5, 17 and 50 mT for times of 2 and 4 hours, but increased the enzymatic activity represented in the dinitrogenase together with an increase in ATP, which is attributed to the stress generated by the magnetic field.

As observed, the great majority of the experiences has presented a growth of these induced by the field, but, nevertheless there are cases where this tendency has not been given (Fojt et al. 2004, Kohno et al. 2000, Strašák et al. 2002), this being due to different variables such as the magnitude of the magnetic field, the exposure time and the type of microorganism (Mittenzwey et al. 1996, Yavuz & Çelebi 2000).

According to the above, this paper presents the results obtained after exposing the microorganisms present in a sample of activated sludge to magnetic fields of 5, 10 and 20 mT for periods of 30, 60 and 120 minutes on a laboratory scale, seeking to find the behavior (inhibition or stimulation) of the microorganisms present in sludge.

MATERIALS AND METHODS

Collection and Preservation of Activated Sludge

1.5 L of the activated sludge with characteristics of 9575 mg/L of volatile suspended solids (VSS) and 1000 mg/L of chemical oxygen demand (COD) was obtained. To maintain said conditions, every 12 hours 1.5 L of a molasses solution with a concentration of 0.6 g of molasses for each litre of water was added. Additionally, the sludge was constantly aerated through an engine.

Construction and Generation of Magnetic Fields

To achieve magnetic fields of 5, 10 and 20 mT, a coil of 8 centimetres in height with a diameter of 3 centimetres and around 1200 turns of copper wire was required. Sources were used that worked with a current intensity of 0.3, 0.5 and 1.1 amperes. These were connected to the coil by means of a PASCO sensor, model CI-6520A.

Identification of Microorganisms in Activated Sludge

To identify the microorganisms present in the activated sludge, 10 mL of activated sludge was added to a beaker containing 90 mL of 0.9% saline solution. Subsequently, serial dilutions were made to distinguish viable microorganisms, where 1 mL of the previous solution was measured with a pipette and added to 9 mL of saline in a test tube and so on until achieving dilution factors of 10^{-2}, 10^{-3} and 10^{-4}. After making the dilutions, 0.1 mL was seeded on nutrient agar and on Sabouraud dextrose agar, incubated for 24 to 48 hours for bacteria and 3 to 4 days for fungi. This process was performed in triplicate. The dilution factor that allowed the best formation and identification of colony-forming units was identified. The colonies formed of bacteria and fungi were characterized macroscopically and microscopically, by means of the Gram stain and lactophenol blue staining methods.

Experimental Design

The generation of the low-frequency magnetic field was carried out by means of a conventional coil, connected to three batteries to vary the magnetic field density until obtaining values of 5, 10, and 20 mT, referring to low-frequency magnetic fields. Subsequently, 10 mL of activated sludge was introduced by means of a test tube into the coil where the magnetic field is uniform, with exposure times of 30, 60, 120 minutes in reference to that reported by Filipiè et al. (2012), since these authors indicated that they are the optimal times for the growth of microorganisms.

At the end of the exposure time to the magnetic field, the samples were subjected to the dilution factor established in the identification of microorganisms in the activated sludge. Later they were seeded on Petri dishes with nutrient agar and Sabouraud dextrose agar, then the colonies formed between the samples were compared, morphologically and by colony-forming units. The process was carried out twice for each magnetic field, varying the exposure time, indicating that 6 tests were performed for each magnetic field, giving a total of 36 tests.

RESULTS

Below are the results obtained for each dilution factor performed, where a count of the colonies, a morphological description and a microscopic determination were carried out, which were subsequently compared to find the closest type of microorganisms with which they have more similarity and the dilution factor that would allow the best generation and visualization of the colonies to form after exposure to the magnetic field.

Determination of Colonies and Optimal Dilution Factor

Determination of bacterial colonies and optimal dilution factor, nutrient agar: The results obtained with the dilution factor of 10^{-2}, 10^{-3} and 10^{-4} for bacteria in the nutrient agar

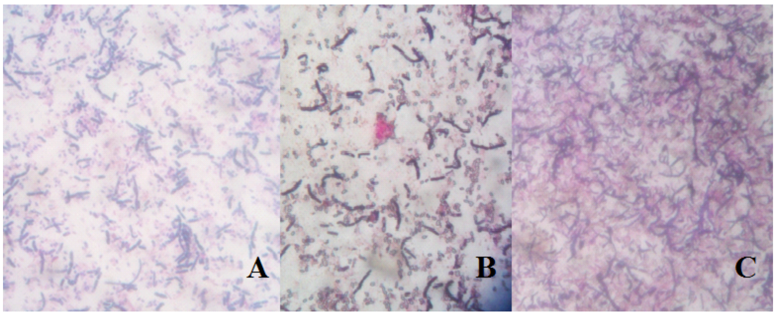

Fig. 1: Microscopy of a sample of the bacterial colonies formed on nutrient agar, 100X magnification, A: 10^{-2} dilution, B: 10^{-3} dilution, C: 10^{-4} dilution.

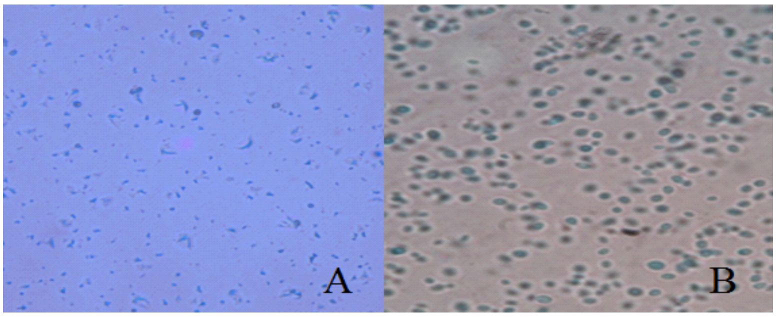

Fig. 2: Microscopy of a sample of the fungal colonies formed on Sabouraud dextrose agar, dilution 10^{-3}, A: 10X magnification, B: 40X magnification.

presented in their great majority large colonies, whose size was between 0.2 to 1.7 cm in diameter, with white colour, irregular shape and edges, smooth surface and soft consistency. A bright white protuberance was also observed in the center of the colonies.

On the other hand, when performing the Gram stain and taking the samples under a microscope, Gram-positive bacilli were found mostly, some in long chains, others in isolation or in parallel pairs, Gram-negative cocci in the form of staphylococci were also found or isolated, as seen in Fig. 1.

After performing the colony count in each of the repetitions made for each dilution factor, the average number of colonies was determined for each dilution. It was also observed that the colonies decreased in size and proportion from one dilution factor to another. Therefore, for the choice of the dilution factor that would allow in the magnetic field tests the best identification and formation of bacterial colonies, two requirements were taken into account: mainly that they were between 30 and 300 colonies per Petri dish and that the morphology of the colonies had a great similarity in size and shape.

According to the above, it was determined for bacteria in nutrient agar to perform a dilution factor of 10^{-2} after exposure to the magnetic field since in this dilution factor the colony count is within the required range, since it has an average of 41 colonies, and in this dilution factor colonies can be better identified both macroscopically and microscopically.

For the colonies that grew in the nutrient agar and comparing them with some technical data sheets of the agar, it could be deduced that the genus of the bacteria closest to that obtained in the laboratory refers to the genus *Bacillus*, specifically *Bacillus cereus*. Concerning Gram-negative cocci, the species of *Staphylococcus aureus* was found, which despite being Gram-positive cocci, can be Gram negative after a certain amount of time. However, the colonies have a yellow pigmentation, which was not observed in the Petri dishes.

Determination of fungal colonies and optimal dilution factor, Sabouraud dextrose agar: For the dilution factors of 10^{-2}, 10^{-3} and 10^{-4} for fungi, on the Sabouraud dextrose agar in its entirety medium colonies were present. The size of the colonies did not exceed 0.5 cm, with bright whitish colour, circular shape, regular edge, smooth surface and soft consistency.

Table 1: Count of bacterial colonies formed on nutrient agar, dilution of 10^{-2}, after exposure of activated sludge to a magnetic field of 5, 10 and 20 mT for 30, 60 and 120 minutes.

	Colonies formed		
	Magnetic field		
Time	5 mT	10 mT	20 mT
30 min	49	48	53
	47	46	62
Average	48	47	58
60 min	41	76	23
	39	62	22
Average	40	69	23
120 min	54	46	31
	45	54	30
Average	50	50	31

Table 2: Average count of baterial colonies (in CFU/mL) formed on nutrient agar, dilution of 10-2, for activated sludge control samples and samples after exposure to a magnetic field of 5, 10 and 20 mT for, 30, 60 and 120 minutes. The control samples were not exposed to a magnetic field

Magnetic field	Time		
	30 min	60 min	120 min
5 mT	48000	40000	50000
10 mT	47000	69000	50000
20 mT	58000	23000	31000
Control	41000	41000	41000

Table 3: Bacterial growth rate, as percentage of average count relative to control sample, formed on nutrient agar, dilution of 10^{-2}, after exposure of activated sludge to different magnetic fields for different periods of time.

Magnetic field	Time		
	30 min	60 min	120 min
5 mT	17%	0%	22%
10 mT	15%	68%	22%
20 mT	41%	-44%	-24%

When staining with lactophenol blue and taking the samples under a microscope, mostly microorganisms of circular or oval shape were found, without clearly defined filaments or hyphae, most of them isolated. For the case of fungi, the sample with 10^{-3} dilution factor was viewed in a magnification of 10X and 40X to observe some type of characteristics that will help its later identification, as seen in Fig. 2.

After performing the colony count in each of the repetitions made for each dilution factor, the average number of colonies was determined for each dilution. For the choice of the dilution factor that would allow in the magnetic field, tests the best identification and formation of fungal colonies, the same requirements as for bacteria were taken into account. Therefore, it was determined for fungi on Sabouraud dextrose agar to perform a dilution factor of 10^{-3} after exposure to the magnetic field.

For the identification of the fungus in Sabouraud dextrose agar, we again compared the colonies that grew on the agar with some technical data sheets of the agar, where the resulting colonies are presented after seeding. It could be deduced that the species closest to that obtained in the laboratory refers to *Candida* spp.

Calculation of Microorganisms Exposed to Magnetic Fields

Seeding on nutrient agar, dilution factor 10^{-2}, after the application of magnetic fields: In Tables 1 and 2, the results obtained after the exposure of the activated sludge to the different magnetic fields for the different times are presented. In the majority of the cases, an increase in the bacterial colonies was obtained compared to the average of 41 colonies obtained in the dilution factor of 10^{-2} of the activated sludge without exposure to magnetic fields.

This allowed calculating the percentage of growth of microorganisms that was exposed to a magnetic field for different periods of time (Table 3). This highlights those whose values are negative, which indicates that there was a decrease in the amount of microorganisms present. The sample of activated sludge exposed to a magnetic field of 5 mT for 60 minutes did not have any variation outside the range of the control samples, therefore its growth rate is 0%, however mathematically it had a growth rate of -2% because the calculations were made with the calculated average.

For the bacteria after being subjected to a magnetic field of density of 5 mT during times of 30, 60 and 120 minutes, the morphology was maintained, however a greater number of colonies were presented during the exposure times of 30 and 120 minutes, while for the time of 60 minutes it remained within the control range. The exposure time of 120 minutes presented the highest percentage of growth with a value of 22%, followed by an exposure time of 30 minutes with a growth rate of 17%.

For the bacteria after being subjected to a magnetic field of density of 10 mT during times of 30, 60 and 120 minutes, a larger number of colonies were present during all exposure times, whose averages were found outside the control range. In this magnetic field, at 60 minutes of exposure is the highest growth rate of 68%, followed by 22% for an

Table 4: Value of colonies formed by Petri dish in Sabouraud dextrose agar, in dilutions of 10^{-3}, with magnetic fields of 5, 10 and 20 mT at exposure time of 30, 60 and 120 minutes.

Time	Magnetic field 5 mT	10 mT	20 mT
30 min	>300	123	221
	>300	115	245
Average	NA	119	233
60 min	>300	260	210
	>300	>300	224
Average	NA	NA	217
120 min	>300	239	156
	>300	233	179
Average	NA	236	168

Table 5: CFU/mL of fungi present in the activated sludge and samples exposed to magnetic fields of 5, 10 and 20 mT during times of 30, 60 and 120 minutes.

Magnetic field	Time 30 min	60 min	120 min
5 mT	3000000	3000000	3000000
10 mT	1190000	3000000	2360000
20 mT	2330000	2170000	1680000
Control	2000000	2000000	2000000

Table 6: Percentage of growth of fungi present in the activated sludge exposed to magnetic fields of 5, 10 and 20 mT during times of 30, 60 and 120 minutes.

Magnetic field	Time 30 min	60 min	120 min
5 mT	50%	50%	50%
10 mT	-41%	50%	18%
20 mT	17%	9%	-16%

exposure time of 120 minutes and 15% for 30 minutes. Comparing this field with the results obtained in the magnetic field of 5 mT, a similarity is found in the times of 30 and 120 minutes in the increase of CFU/mL.

Finally, for the magnetic field of density of 20 mT during times of 30, 60 and 120 minutes, the bacterial growth was presented only in the exposure time of 30 minutes with a growth rate of 41%, while for the exposure times of 60 and 120 minutes the number of colonies formed decreased. This indicates that there is an inhibitory effect on growth after being exposed to samples at times greater than 30 minutes, reaching inhibitions of 44% and 24%.

Seeding on Sabouraud dextrose agar, dilution factor 10^{-3}, after the application of magnetic fields: After the exposure of the activated sludge to the different magnetic fields for the different times, the majority of the cases obtained an increase in the colonies compared to the average of 200 colonies obtained in the dilution factor of 10^{-3} of the activated sludge without exposure to magnetic fields.

For the fungi after being exposed to a magnetic field of density of 5 mT during times of 30, 60 and 120 minutes, the morphology was maintained and a greater number of colonies were presented during all exposure times, whose averages were found outside the control range, and even the colony count exceeded 300, as given in Table 4. For a magnetic field of 5 mT, an increase can be observed in reference to the control with growth rates greater than 50% in all exposure times, because during the colony count they exceeded 300 colonies, therefore to have a reference value was chosen to maintain 3000000 CFU/mL as the maximum limit. However, these represent higher values, indicating that there are no differences for fungi when exposed to a time of 30, 60 or 120 minutes (Tables 5 and 6).

For the magnetic field of density of 10 mT during times of 30, 60 and 120 minutes, a greater number of colonies were presented during the exposure times of 60 and 120 minutes, however, for an exposure time of 30 minutes there was a decrease in the colonies compared to the control ranges. The growth percentage in the exposure times of 60 and 120 minutes was 50% and 18% respectively, clarifying that for the exposure time of 60 minutes, one of the Petri dishes exceeded 300 colonies, therefore the value presented as average was the cap of 3000000 CFU/mL, which indicates that the growth rate of 50% remains as the maximum presented. For the exposure time of 30 minutes there was an inhibitory effect on the fungi of 41%.

For the fungi after being exposed to a magnetic field of density of 20 mT during times of 30, 60 and 120 minutes, a greater number of colonies was presented during the exposure times of 30 and 60 minutes, whereas for an exposure time 120 minutes there was a decrease in the colonies compared to the control ranges. It is important to mention that in this field the lowest percentages of growth were presented, these being 17% and 9% respectively. Finally, for the exposure time of 120 minutes to a magnetic field, an inhibitory effect of 16% was found.

CONCLUSIONS

By exposing the microorganisms present in an activated sludge to magnetic fields of 5, 10 and 20 mT, a greater formation of microorganisms is generated in most of the magnetic fields at the time of exposure, although the amount of microorganisms is also decreased in specific cases, as they are for bacteria at 20 mT for 60 and 120 minutes, and for fungi at 10 mT for 30 minutes and 20 mT for 120 minutes.

In bacteria, the greatest growth is generated by exposure to a magnetic field of 10 mT for 60 minutes, however for times of 30 and 120 minutes in this same field there is an increase in microorganisms, similar to those that can be obtained by decreasing the magnetic field to 5 mT.

For fungi, the greatest growth occurs with a magnetic field of 5 mT during all exposure times. In the same way, the greatest decrease of microorganisms occurs in a field of 10 mT for 30 minutes of exposure. The exposure of both fungal and bacterial microorganisms to a magnetic field of 20 mT during an exposure time of 120 minutes generates a decrease in the microorganisms present in the activated sludge.

For an activated sludge, the proliferation of the organic matter-degrading bacteria is required at the same level as the filamentous microorganisms. However, in the case of the activated sludge that was exposed to the magnetic fields, in the identification of microorganisms, neither the bacteria nor the fungi were found to be filamentous. Therefore, in order for there to be degraders of organic matter, it is required that there is an increase in the microorganisms present in the activated sludge for which the most optimal magnetic field and exposure time would be of 10 mT for 60 minutes, because they have a growth rate greater than 50% for both bacteria and fungi.

By means of macroscopic and microscopic identification, it can be concluded that *Bacillus* spp. and *Candida* spp. are representative microorganisms of the activated sludge and that their magnetic stimulation is beneficial to increase the quantity of microorganisms present in the biological treatment, thus improving the treatment conditions, because they are degrading and at the same time filamentous.

In the case of an activated sludge containing filamentous fungi, the magnetic field and the optimal exposure time would be 10 mT for 30 minutes, since it stimulates bacterial growth while in turn decreasing fungal growth, if and only if these exceed in concentration to the degrading microorganisms, avoiding filamentous bulking. However, as reported in the literature, the effect of the magnetic field on microorganisms depends on the genus or species, because some microorganisms are more susceptible to this type of exposure than others.

In the case of an activated sludge containing fewer filamentous microorganisms, in this case fungi, the magnetic field and the optimum exposure time would be 5 mT for 60 minutes, since it stimulates fungal growth while in turn does not generate changes in the growth of the bacteria, if and only if they exceed in concentration to the filamentous microorganisms, avoiding the problem of a lumpy or pinpoint mud.

The application of magnetic fields in the biological treatment of activated sludge can become a solution to the control of bulking or lumpy mud due to the ease that it can have to inhibit or increase the growth of microorganisms, depending on what is required.

Exposing the microorganisms of an activated sludge to magnetic fields of low frequency can be beneficial for the biological process of wastewater treatment. However, it requires greater insight due to the susceptibility that each microorganism has to the exposure to the magnetic fields and its function or presence within the treatment.

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