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Remediation

Removal of hydrogen sulfide by microorganisms on activated sludges in the wastewater of food industry

Remoción de ácido sulfhídrico por microorganismos sobre lodos activados en aguas residuales de la industria alimenticia

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Abstract

The importance of this work is the implementation of a natural treatment with low environmental impact. The objective was to evaluate effective microorganisms for the removal of hydrogen sulfide on activated sludges of the industrial wastewaters. For the experimental part of the research a biofilter was built with effective microorganisms, activated sludge and filter material. 21 liters of activated EM were inoculated on sludges for over 3 weeks, controlling parameters such us pH, temperature, humidity, oxygen and sunlight, guaranteeing the optimal conditions for the develop of photosynthetic bacteria, lactic acid and yeast. Sampling of wastewater was carried out in the closest manhole to the point of discharge of the sewage system of La Iberica and for its analysis at the biofilter output, taking a sampling with three repetitions each week, for 21 days, to know the initial, intermediate and final concentration of H_2S and the removal percentage reached during the treatment. Research findings showed that the effective microorganisms increased the microbial load of the endogenous microorganisms in the sludge, obtaining a better growth of the biofilm and achieving the reduction of the H_2S concentration from 3.68 mg/L to 0.45 mg/L, reaching a removal percentage of 70.35%. This work analyses that to a greater retention times, a better performance of the effective microorganisms is given. The use of effective microorganisms in the removal of H_2S is profitable since the time of action is short and the costs of application are low.
Keywords: Removal, Hydrogen sulfide, efficient microorganism, sampling, activated sludge, biofilm.

Resumen

La importancia del siguiente trabajo radica en implementar un tratamiento de remoción de H_2S con un bajo impacto ambiental. El objetivo fue el de evaluar microorganismos eficientes para la remoción de ácido sulfhídrico sobre lodos activados de las aguas residuales industriales. Se construyó un biofiltro con microorganismos eficientes, lodo activado y material filtrante. Se inocularon durante tres semanas en lodos 21 litros de microorganismos eficientes activado, controlando parámetros como pH, temperatura, humedad, oxígeno y luz solar, garantizando las condiciones aptas de desarrollo de bacterias fotosintéticas, ácido lácticas y levaduras. El muestreo del agua residual se realizó en la caja de revisión más cercana al punto de descarga al alcantarillado de La Ibérica, y para su análisis a la salida del biofiltro, tomando una muestra con tres repeticiones cada semana, durante 21 días, para conocer la concentración inicial, intermedia y final de H_2S y el porcentaje de remoción alcanzado en el tratamiento. Los resultados de la investigación dictaminaron que los microorganismos eficientes aumentaron la carga microbiana de los microorganismos endógenos en el lodo, consiguiendo un mejor crecimiento de la biopelícula y logrando la reducción de la concentración de H_2S de 3,68 mg/L a 0,45 mg/L, alcanzando un porcentaje de remoción del 70,35 %. El presente trabajo analiza que a mayores tiempos de retención se da una mejor actuación de microorganismos eficientes en el tratamiento. La utilización de microorganismos eficientes en la remoción de H_2 S, resulta rentable por sus tiempos cortos de actuación y bajos costos de aplicación.
Palabras claves: remoción, ácido sulfhídrico, microorganismos eficientes, muestreo, lodo activado, biopelícula.

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1 Introduction

Hydrogen sulphide is generated from a biological reaction in sewage systems or wastewater treatment plants, being the cause of the corrosion of sewerage systems (Bas, 2017). Wastewater with pH ranges below 5.0 generate an environmental impact, since when discharged directly into rivers they cause bad odors and the loss of their native biota (Fernández-Alba et al., 2006).

Most of the H_2S generation occurs in the mud layer that forms on the walls of the pipe or in the deposits that form at the base of the pipe. If the water served contains little or no dissolved oxygen, the diffusion of H_2S occurs due to the existing anaerobic conditions (Arriagada Monreal, 2008).

Currently in Ecuador the processes used for the removal of hydrogen sulfide in wastewater involve chemical treatments to a large extent such as the use of coagulants, chlorinated products, photocatalysis with light, a semiconductor (TiO_2) as a catalyst and an oxidizing agent such as hydrogen peroxide (H_2O_2) that involve high costs and less accessibility to all sectors (Varela Reyes, 2013). However, nowadays, there are other biotechnological methods that involve the use of microorganisms for their biological treatment, seeking to increase the capacity to implement processes that are friendlier to the environment (Szymanski and Patterson, 2003).

The importance of this research lies in using efficient microorganisms as an accessible alternative to the problem of pollution in the area of biological treatment of wastewater, not only because they involve economic prices but also because of their short removal times.

Efficient Microorganisms Technology ™ (EM ™) is a natural, lower impact treatment developed by Prof. Teruo Higa at the University of Ryukyus, Okinawa, in southern Japan, starting in 1982 (EEAI-TAJ, 2013). Originally, it was used as an alternative for chemical fertilizers and pesticides, however, the use of EM™ Technology, in the last two decades, has expanded from agriculture to the treatment of water and effluents, odor control and innumerable treatments industrial (Madigan et al., 2004).

The present work aspires to be a document with practical and theoretical contribution for future research in the evaluation and application of efficient microorganisms for the reduction of hydrogen sulfide and other pollutant compounds of industrial wastewater in our country.

When the present research work is developed, the direct beneficiaries will be mainly the industries, by obtaining a biological treatment system of lower cost that allows them to reduce a greater percentage of hydrogen sulfide before its discharge, in less time and fulfilling the current environmental norm.

2 Materials and method

2.1 Techniques and data tabulation tools

The statistical analysis was carried out in the SPSS program, by nonparametric Kolmogorov-Smirnov tests of a sample and one-way ANOVA, allowing the quantitative data to be analyzed and interpreted. According to the Environmental Quality and Effluent Discharge Standard: Water Resource, Book VI Annex 1, the maximum permissible limit of H_2S for fresh, cold or warm water, and in marine and estuary waters is 0.0002 mg/l.

For the quantification of H_2S, the methylene blue method (Standard Methods 4500-S2-D/HACH 8131) was used, based on the reaction of hydrogen sulphides and acid-soluble metal sulfides with N, N-dimethyl-p- Phenylenediamine sulfate to produce methylene blue. The intensity of the blue color is proportional to the concentration of sulfur present in the sample. The reading is made in a spectrophotometer at 664 nm.

2.2 Stages of the research

The statistical analysis was carried out in the SPSS program, by nonparametric Kolmogorov-Smirnov tests of a sample and one-way ANOVA, allowing the quantitative data to be analyzed and interpreted. According to the Environmental Quality and Effluent Discharge Standard: Water Resource, Book VI Annex 1, the maximum permissible limit of H_2S for fresh, cold or warm water, and in marine and estuary waters is 0.0002 mg/l.

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2.2.1 First stage: Process of activation and inoculation of efficient microorganisms

The microorganisms of the product EM·1, is composed of photosynthetic bacteria, lactic acid bacteria and yeasts, which are in a latency state, for the activation the EMRO Technical Guide of EM Technology was followed (EMPROTEC, 2016) AGEARTH being its official representative in Ecuador.

At 7 days after fermentation, the aforementioned microorganisms are activated and ready to be used as long as they have a pH below 4.00, a pleasant bittersweet odor and a change in coloration from dark brown to orange-colored coffee (EMPROTEC, 2016).

The microbiological characterization of the sludge used as a substrate was carried out before and after incorporating the efficient microorganisms into the unit of analysis, to describe the concentration of both pathogens: molds and yeasts, fecal and total coliforms and aerobic mesophilic microorganisms present (Galvis Toro and Rivera Guerrero, 2013). For the evaluation of these microbiological parameters the same methodology established in the standard was used, varying only in the culture media, the plates used, and type of planting and incubation temperature (INEN, 2013).

During the three weeks of treatment, 1 liter of Efficient Microorganisms activated every 6 hours in the mud was inoculated and stirred every week to allow its aeration.

2.2.2 Second stage: Second stage: Sampling of wastewater before treatment

The waste water intake was carried out in the check box closest to the point of discharge to the sewage system of the food processing industry and its transfer was immediate for its subsequent refrigeration and storage.

For initial laboratory analyzes, 3 samples were taken, each with 3 replicates, from a total of 15 samples collected, before passing the wastewater through the unit of analysis to know the initial concentration of H_2S present in the waters to be treated (Coronel Pazmiño, 2015).

The sampling process was carried out in three phases, in the first phase, a sample of 3 liters was collected from 15 samples, giving us a total of 45 liters, in the second phase we obtained 15 liters that were collected for 5 days, in the third phase, like the second, 15 liters were collected, in 5 days of sampling, giving us a total of 75 liters of industrial wastewater to be used in the investigation.

2.2.3 Third Stage: Construction of the analysis unit (Biofilter)

The unit was built based on the established dimensions of a laboratory-scale biofilter designed by (Higuera, Arroyave and Flores, 2009) to reduce the rate of chromium contamination generated in the leather tanning industries. With this reference, we proceeded to the construction of a biofilter with the following dimensions, as shown in Figure 1: height 37.2 cm, length 32 cm and width 31.3 cm.

The material used was 8 mm thick transparent glass with an 8 cm diameter hole in the center of its base for the water outlet. A mesh was also placed to prevent the passage of solids, a stopcock to control the flow of water, four hoses with holes placed in each corner of the biofilter for the aeration of the sludge and a hose with symmetrical holes for the water to be Distribute to the entire unit of analysis containing activated sludge, EM (Efficient Microorganisms) and a filter material.

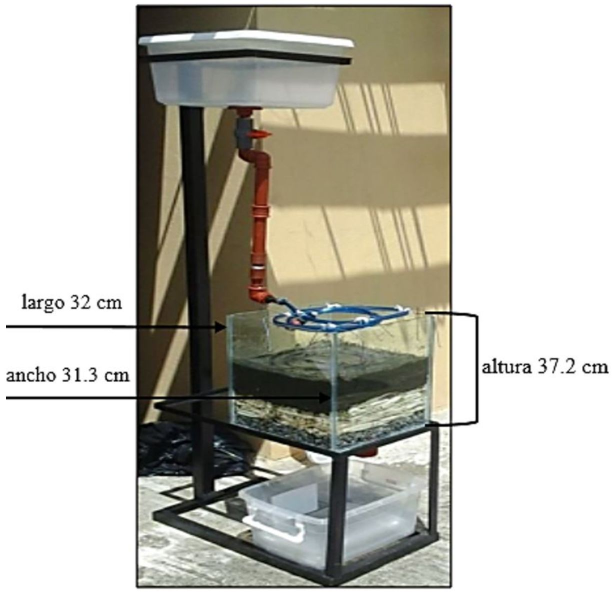

Figure 1. Biofilter at Laboratory scale.

2.2.4 Fourth stage: Establishment of suitable conditions for efficient microorganisms in the Biofilter

Once the analysis unit was built, the control variables suitable for the development and multiplication of efficient microorganisms were monitored, being considered as the most important pH, temperature, humidity.

pH control

The efficient microorganisms contain photosynthetic bacteria, lactic acid and yeasts that need an acidic pH for their correct development and multiplication (Moreno and Velarde, 2016). Therefore, a control was carried out before and during the treatment to ensure that the pH of the sludge is maintained between a constant acidity ranges to ensure the survival of the microorganisms within the biofilter.

The pH reading was taken with the help of pH indicator paper strips. This parameter was monitored by immersing the strip on a fresh aqueous mud sample (every week) and comparing the color obtained with the scale contained in the package to compare its degree of acidity or alkalinity (UTE, 2014).

Temperature control

Temperature monitoring was carried out daily with the help of a digital thermometer, taking into account that at high temperatures the microorganisms act with greater efficiency, so this parameter must oscillate between 30-45 °C throughout the treatment (Lázaro, 2014). To maintain adequate stability within the unit of analysis, and considering that the climate of the place is variable, a greenhouse was installed, which helped to control this parameter (Galvis Toro and Rivera Guerrero, 2013).

Humidity control

This parameter is important since the sludge must always be kept moist inside the biofilter, since if the humidity drops, the microorganisms do not develop because they do not have enough water for their metabolism, so it is considered that the humidity must be between 50 -70 % (Salinas Carrillo and Huacollo Álvarez, 2011).

To determine the humidity, the empirical method of the fist test was considered (Novoa et al., 2008). The technique tells us that if the tight substrate does not take the shape of a closed fist and does not drip, the approximate humidity is less than 70 %, on the contrary if by grabbing a quantity of the substrate that reaches with the fist of a hand, they drain more of 10 drops in 1 minute, means that the humidity is about 80 %.

Taking into account the previously detailed technique, a mud sample was taken from the center of the biofilter and it was strongly pressed, as a continuous stream of water emerged, the sample was considered to be between a correct humidity range (>70 % approximately).

2.2.5 Fifth stage: Sampling of treated water

During the treatment with efficient microorganisms, three samples of filtered water were taken, each with three repetitions. Sampling was carried out in 1-liter plastic bottles, labeled as follows: M1 (EM), for sample 1 of the treatment with efficient microorganisms; M2 (EM), sample 2 of the treatment with efficient microorganisms and M3 (EM), sample 3 of the treatment with efficient microorganisms (Coronel Pazmiño, 2015).

The first sampling was done in week one of the treatment, the sample of filtered water was taken, with three repetitions to know the concentration of hydrogen sulfide and additional monitoring of pH, conductivity and turbidity were carried out, to evaluate the incidence of said parameters in the reduction of H_2S. The second and third sampling was done every eleven days, since at this time the microorganisms stabilize (Jusoh, Manaf and Latiff, 2013).

3 Results and discussion

3.1 Microbiological characterization of activated sludge

As shown in Figure 2, an analysis was performed before inoculating the efficient microorganisms in the mud (day 1) that allowed to identify the presence of aerobic mesophiles, total coliforms, fecal coliforms, molds and yeasts, in the same way an analysis after 15 days of having started with the treatment, in order to evaluate the behavior of the EM (Efficient Microorganisms) before the changes of the control variables. The result is shown in Table 1.

Table 1. Microbiological parameters evaluated in the activated sludge.

	(UFC/mL)				
Time (days)	Aerobic mesophiles	Total coliforms	Fecal coliforms	Mold	Yeasts
1	580	340	Presence	Presence	9200
15	5200	110	Absence	Absence	10900

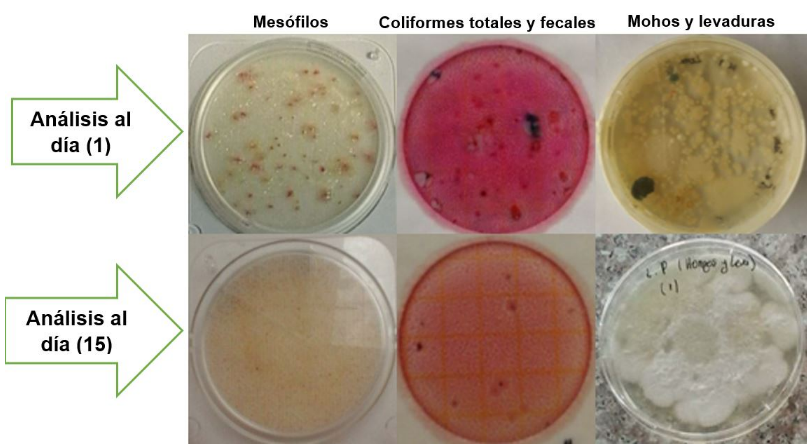

Figure 2. Growth in plaque of microorganisms.

3.2 Control of conditions suitable for the activation and development of efficient microorganisms in activated sludge.

Figure 3 shows the results of the behavior of pH and temperature during the activation and the period of 21 days in which the control was carried out for its adaptation. As for the activation, an inversely proportional relation is fulfilled, since the more days pass for its fermentation the pH decreases. From the fourth day the fermentation is accelerated showing a significant pH decrease of 4.55 while on the seventh day it reaches a value of 3.33. Therefore, the Efficient Microorganisms product on day 7 complies with a range of acidity lower than 4.00 considered as optimal for its activation and suitable for use (Organic Fruits, 2016).

The temperature at the first days indicates variations of 28 to 30°C, while over time, variations of 32 to 35°C are visualized, as shown in Figure 3. The trend line shows that the temperature increases and decreases at a constant rate. Therefore, despite the external environmental variations, a tendency to maintain stable conditions of warm temperature of around 30 - 35 °C during the treatment is observed.

In terms of pH, it tends to maintain a constant acidity range, comprised between a value of 4-5, with a linear trend during the 21 days of treatment, which makes the substrate an adequate medium for the growth of photosynthetic microorganisms, lactic acid and yeasts (García et al., 2008).

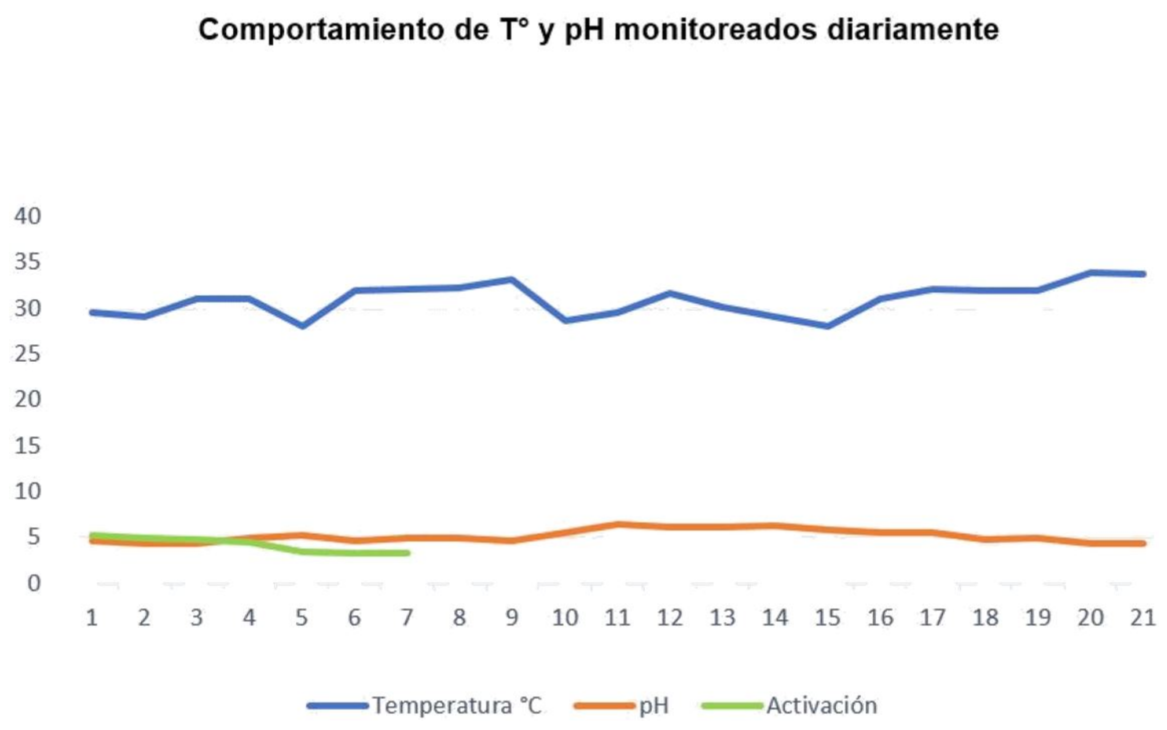

Figure 3. T° and pH behavior monitored daily.

4 Análisis de la concentración de ácido sulfhídrico

In order to verify if the treatment with efficient microorganisms (factor), during the three weeks, had favorable results in the removal of hydrogen sulfide from the wastewater, a Completely Random Design was performed in the SPSS program. (Table 2).

Table 2. Anova analysis of a factor.

ANOVA of a factor

		Sum of squares	gl	Quadratic mean	F	Sig.
H2S	Inter-groups	11,416	2	5,708	24,566	0
	Intra-groups	1,394	6	0,232		
	Total	12,81	8			
pH	Inter-groups	6,883	2	3,442	6883,4	0,000
	Intra-groups	0,003	6	0,001		
	Total	6,886	8			
Conductivity	Inter-groups	16,692	2	8,346	23473,63	0,000
	Intra-groups	0,002	6	0,000		
	Total	16,694	8			
Turbidity	Inter-groups	18716,67	2	9358,333	1238,603	0,000
	Intra-groups	45,333	6	7,556		
	Total	18762	8			

In this case, Anova of a factor is determined by the significance value, which is approximately 0 in the analysis of the variables H_2S (p=0.001), pH (p=0.000), conductivity (0.000) and turbidity (p = 0.000); therefore, having a result of $p < 0.05$, the null hypothesis is rejected and the alternative hypothesis is accepted, which shows that there are differences between the treatment weeks, with at least a couple of comparisons of all possible ones. This means that the weeks influence the concentration of hydrogen sulfide, pH, conductivity and turbidity in the wastewater to be treated.

Table 3. Descriptive analysis of the variable H_2S by weeks of treatment with EM.

Descriptives
H2S

	N	Mean	Typical desviation	Typical error	Confidence interval for the 95% mean		Minimum	Maximum
					Lower limit	Upper limit		
1	3	3,170	0,815	0,470	1,145	5,194	2,237	3,687
2	3	1,426	0,150	0,086	1,052	1,800	1,274	1,570
3	3	0,446	0,100	0,058	0,196	0,696	0,346	0,541
Total	9	1,681	1,265	0,421	0,708	2,653	0,345	3,688

Table 3 shows the means of H_2S in each week of treatment with efficient microorganisms, in which it is observed that they have differences of one week in relation to the other, being the average of week 3 that presents a value minor compared to week one and two. Regarding the standard deviation, similar occur in week 2 and 3, with the exception of week one, where a lower standard deviation is observed. The 95 % confidence interval shows estimates by intervals of the location of the true population mean of H_2S in relation to each of the weeks of treatment, with the minimum and maximum values of the time in weeks that the treatment was carried out.

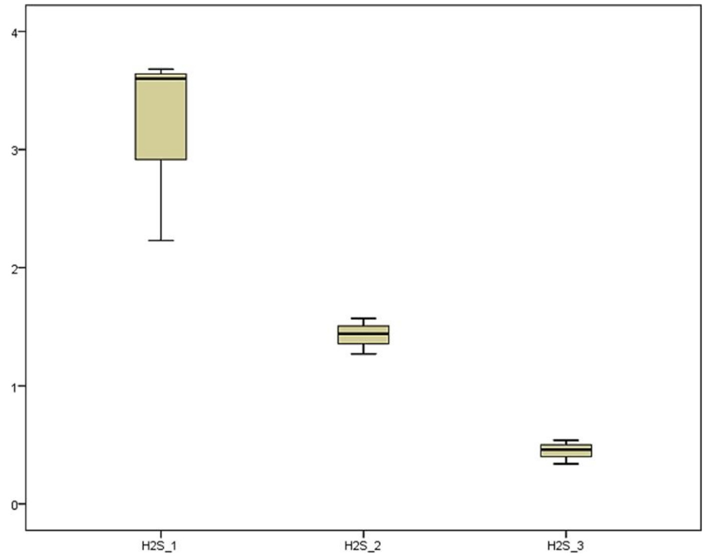

Figure 4. Mean concentration of H_2S during three weeks of treatment with Efficient Microorganisms.

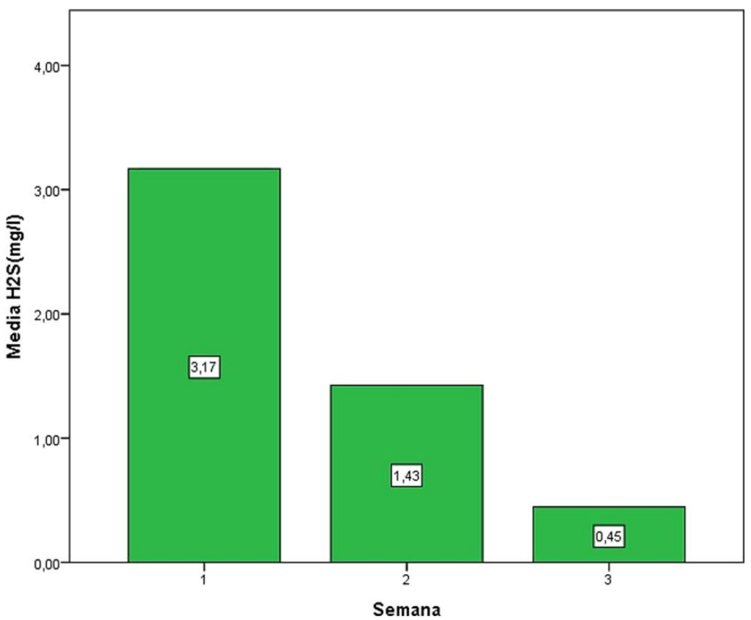

Figure 5. Media de la concentración de H_2S durante tres semanas de tratamiento con Microorganismos Eficientes.

Figure 5 shows the mean concentration of H_2S analyzed in wastewater from a food processing industry, in relation to three weeks of treatment with efficient microorganisms.

In week three, a value of 0.45 mg/L of concentration of H_2S (TRH=12 hours) is presented, considered to be significantly lower compared to week one and two, with values of 3.17 and 1.43 mg/L respectively, with a TRH of 6 hours in the two weeks.

In the three weeks of treatment there was a reduction in the concentration of H_2S, however, in week one its reduction was low, because the microorganisms were populating and adapting to the environment (Rehman, Farooqi and Ayub, 2009), being that in week two there were already changes in the higher concentration reduction, week three was the one that had the greatest impact, managing to reduce from an initial concentration of 3.17 mg/L to a final concentration of 0.45 mg/L, obtaining a removal percentage of 70.35%; which indicates that the biofilter in the course of time reaches greater stability and better microbial growth in the biofilm (Welter et al., 2010). This is particularly evident with the use of sulfate-reducing bacteria, photosynthetic bacteria, lactic acid and yeast, which increase their microbial load thanks to the native organic matter of the activated sludge, being very viable so that said organic matter is retained in the layers of the biofilter, avoiding being dragged when passing a new load of residual water through the biofilter.

Additional parameters such as pH, conductivity and turbidity were directly related to the reduction of hydrogen sulphide. As for the pH, it has a directly proportional relationship with the percentage of H_2S removal; as the percentage of removal increases, the pH decreases (Cardona Gómez and García Galindo, 2008).

When analyzing the conductivity, it showed an increase because the activities of the efficient microorganisms contribute to the increase of nutrients, ions in solution and heat transmission due to the same environmental temperature (Zamora et al., 2009). The reduction of turbidity determined that, from 15 days after starting the treatment, there is greater activity of the microorganisms, since their adhesion was well developed, which explains the significant reduction of the concentration of H_2S in the week three of the treatment (Peérez Zúñiga, 2016).

5 Discussion and conclusions

It was determined that the microorganisms contained in the EM product (Efficient Microorganisms), act in the activated sludge, increasing the capacity to form the biofilm, and the growth of bacteria responsible for the removal of H_2S in industrial wastewater.

To ensure the development and survival of the efficient microorganisms, on the activated sludge, an acidic pH was controlled during the investigation with ranges between 5.5-6.5, a warm temperature of 30-45 °C, humidity of 60%.

The concentration of hydrogen sulfide in industrial wastewater was analyzed before and after treatment with efficient microorganisms, showing an initial concentration of 3.68 mg/L and a final concentration of 0.45 mg/L, obtaining a higher percentage of removal. in the third week of treatment, with a value of 70.35%. The TRH used in the third week was 12 hours, so it is concluded that managing longer retention times increases the capacity of removal in the treatment.

It was verified that the use of efficient microorganisms in wastewater, for the removal of H_2S, is profitable because of its short acting times and low application costs, besides that, being a natural treatment, it does not cause any damage to the environment.

During the course of the investigation, it was revealed that the sludge used as a substrate for the EM (Efficient Microorganisms), reduced the amount of pathogens and increased the content of nutrients present at the beginning of the treatment, concluding that the residual sludge due to its characteristics can be reused to fertilize the soil in agriculture.

Acknowledgements

We thank the La Ibérica sausage industry for the data provided for the realization of the research, so it is expressly mentioned that there are no commercial interests with the product EM·1, composed of photosynthetic bacteria (10^3), acid lactic bacteria (10^4) and yeast (10^3), used for research since they were simply used for research purposes.

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