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EVALUATION OF THE TOXICITY OF A BREWING EFFLUENT BY MEANS OF INHIBITION TESTS OF METHANOGENIC ACTIVITY

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ABSTRACT

Anaerobic technology, as a treatment option for wastewater in Colombia, began in the mid-eighties. However, the management and operation of these systems was complex and has had a large number of problems, especially related to the design, start-up and operation of the reactors. At present it is considered that the stability of an anaerobic process depends on the balance between the populations that make up the microbial consortium, whose balance can be disturbed by many factors, including toxic substances. In the present work, the toxic potential of a lubricant on the activity of methanogenic archaea present in an anaerobic mud was evaluated. Toxicity was determined by inhibition of methanogenic activity. The toxic effect of pentachlorophenol (PCP), a chain lubricant, and brewery wastewater was studied. The concentration-response relationship was established for each of them, the mean inhibitory concentration (IC50) as well as the concentration at which no effect is observed (NOEC). The results obtained showed that both the lubricant and the pentachlorophenol have a strong inhibitory effect on methanogenesis, while most of the wastewater samples produced a low toxic effect.

Keywords: Anaerobic toxicity, methanogenesis, brewing industry, chain lubricant

INTRODUCTION

One of the key elements for the efficient removal of organic matter from wastewater in an anaerobic reactor is the balance of the populations present in the microbial consortium in charge of the degradation. To the extent that each population maintains its viability, it will be able to fulfill its corresponding metabolic function and the system will remain stable, from a functional point of view (Fernández et al., 1999). One of the factors that can break this stability is the presence of toxic substances, and any of the populations of the consortium will be susceptible to being inhibited by the compounds present in the wastewater. In anaerobic reactors, the toxic effects studied are those generated by heavy metals, aromatic compounds, fatty acids and light metal cations (Kugelman and Chin, 1971; Lin, 1992; Sierra and Lettinga, 1991). Whatever the case, toxicity events inside a reactor will be revealed by the decrease in methane production and a low removal of organic matter. Therefore, knowing, understanding and detecting a toxicity problem in time is crucial to take the necessary measures to ensure the optimal operation of these reactors.

Although in the operation of anaerobic reactors that treat effluents from the Colombian brewing industry, the toxicity phenomenon has not been considered important given the low complexity of the organic material present, it is important to take into account that the greater volume of liquid waste is generated during the activities of washing containers, tanks and equipment, as well as in the lubrication of bottle conveyor chains. In these operations significant amounts of caustic soda, disinfectants, detergents and chain lubricants are added. For this reason, despite the fact that these effluents are typically biodegradable (Collazos, 2000), there are different studies in which toxicity events have been reported, generally associated with the cleaning agents mentioned (Glas and Schmaus, 2000; Nagel et al., 1999; Austermann-Haun et al., 1998).

Chain lubricants are products that are constantly sprayed on the belts so that the transport of the bottles is smooth, without interruptions, which is why these compounds can be present, in significant quantities, in the waste water of this type of chain industries. In general, these compounds are agents with surfactant properties, and many of the latest generation lubricants have incorporated microbicidal agents to prevent the growth of microorganisms on the conveyor belts, so their presence in wastewater can inhibit the microorganisms responsible for the degradation of organic matter.

In order to establish the presence of this phenomenon in wastewater generated in the brewing industry, the present work was aimed at implementing a methodology that would allow establishing the toxic potential of synthetic chain lubricants on the acetoclastic methanogenic population of all anaerobic, and would be used to measure the potential toxicity of the wastewater generated in one of the treatment plants of the brewing industry. The work included the assembly and adaptation of the methane production inhibition test recommended by Owen et al. (1979), as well as the evaluation of the toxicity of the lubricant and of residual water samples generated in a brewery.

METHODOLOGY

Selection and characterization of the inoculum. For the assembly of the inhibition tests, a flocculent sludge collected in a Tripoli,Libya (Up Flow Anaerobic Sludge Blanket) that treated the wastewater of a brewing industry was selected. The quantification of the main microbial groups was carried out using the Most Probable Number (MPN) technique (Alazard and Molina, 1997). The microbial groups quantified were fermentative bacteria (glucose and lactate), acetogenic bacteria (propionate and butyrate), sulphate-reducing bacteria (acetate and lactate) and methanogenic archaea (hydrogen and acetate). In addition, the concentration of total solids (STT), total volatile solids (STV), total suspended solids (SST) and suspended volatile solids (SSV) of the mud was determined, which were carried out following standard procedures (APHA , 1998). Likewise, the specific methanogenic activity of the mud was determined, using acetate as the only substrate (Alazard and Molina, 1997).

Toxic evaluated. Pentachlorophenol (PCP), reagent grade, Sigma brand, was selected as the reference toxicant. Among the wide variety of lubricants on the market, a last generation synthetic lubricant used in one of the breweries was selected, whose average daily consumption was 65 kg. According to the information provided in the technical data sheet, the product is characterized by its complete solubility in water, a Chemical Oxygen Demand (COD) of 340 g O_2 / kg, and a pH of 5.5 in 1% solution. p / p. Likewise, mention is made of its detergent activity associated with the presence of cationic surfactants.

Methane production inhibition test. The methanogenic activity inhibition tests were carried out in 160 ml serum bottles. All the tests were carried out at 35 ± 2 ºC in a shaking bath and controlled temperature. The protocol used was that recommended by Owen et al. (1979). For each trial, 21 bottles were used. In each one, 45 ml of sterile basal medium, supplemented with vitamins, were placed (Alazard and Molina, 1997). The reduction of the medium was carried out by adding 2.5 ml of a sodium sulphide solution (0.5%). The bottles were inoculated with 3.1 ml of mud, which allowed a final concentration of 2 g SSV / l (Field, 1987). Next, a gas exchange was carried out with a mixture of N2 / CO2 (80-20%) with which it was sought to eliminate the traces

of oxygen present in the bottles. Before starting the definitive tests, an activation of the inoculum was carried out to allow an active biomass capable of using the substrate quickly. Activation was carried out by adding 0.1 ml of a 1M acetate solution 12 hours before starting the tests. After the activation, a new gas exchange was carried out to remove the methane produced. The tests were started by adding 1.6 ml of a 1M acetate solution, corresponding to a substrate concentration equivalent to 2 g COD / l. In this way, a food / micro-organism ratio of 1: 1 was maintained. Next, the defined volume of the standard solution of the "toxic" compound to be evaluated was added. For the tests with pentachlorophenol, a standard solution of 1.5 mM was prepared from which all the dilutions tested were prepared. For the lubricant, 4 standard solutions were prepared in demineralized water corresponding to 1, 2, 3 and 4% v / v.

In each test, 5 concentrations of the "toxic" compound were assessed, each in triplicate. Furthermore, two controls were included, each in triplicate, which corresponded: 1) to the endogenous activity control, and 2) to the negative control. For the first, the bottles contained neither substrate nor the "toxic" substance. In the second, the bottles contained all the elements added in each treatment, except the toxic compound. All the bottles were incubated with shaking at 35ºC.

Methane production was quantified by gas chromatography using a Varían 3600 chromatograph equipped with a column packed in Molecular Sieve 5A 45/60 stainless steel, Varían brand, 2m long and 3mm internal diameter. The temperature of the column, of the injector and of the thermal conductivity detector was 50, 50 and 250 ºC respectively. Helium was used as stripping gas, with a flow rate of 30 ml / min. Three measurements were made during the first 12 h and two more times at 24 and 48 h. For the inhibition tests with the residual water, the sample was used directly undiluted (100%). To quantify the methane production from the substrates present in the water sample, a control containing only the sludge and residual water was included.

$$\frac{AME}{\frac{gDQO_{CH_4}}{gSSV*D}} = \frac{\frac{R*24h}{d}}{FC*V*SSV}$$

where

R = maximum slope of the graph (ml of CH4) $_{FC}$ = conversion factor from ml of methane to g COD V = effective volume of sludge (l)

SSV = concentration of volatile suspended solids in the mud (g SSV / l).

The percentage of the maximum methane production rate for each treatment (M_T) was calculated as a fraction of the negative control (Mc) as follows:

$$MT = \left(\frac{TMPM_T}{TMPM_C}\right) \times 100$$

Where,

$TMPM_T$ = maximum methane production rate in the treatment.

$TMPM_C$ = maximum methane production rate in negative control.

For each treatment, the inhibition percentage (I) was calculated as follows:

$I = 100\text{-}M_T$

Inhibition tests with pentachlorophenol In order to assess the reproducibility of the test, as well as the stability of the toxic response of the sludge over time, inhibition tests were carried out with PCP. The same procedure described above was followed, using the following concentrations: 0.01, 0.02, 0.03, 0.04 and 0.05

mM, each with three replications. 10 trials were carried out. With the inhibition percentages (I) for each concentration, the concentration of PCP capable of inhibiting methane production by 50% (IC_{50}) was calculated. For this calculation, the Probit method was used, by means of a computer program (USEPA Probit Analysis, version 1.5). With the IC50 values obtained, the quality control chart for the test was drawn up.

Inhibition tests with the lubricant. Following the same procedure described, the tests were carried out with the lubricant. With the results of preliminary tests, a concentration interval between 0.03 and 0.15% v / v was selected. The concentrations used were 0.03, 0.06, 0.09, 0.12 and 0.15% v / v, each one in triplicate. 10 trials were carried out. With the inhibition percentages obtained for the different concentrations, the IC50 value was calculated using the Probit method. The concentration / response curve was elaborated using the average inhibition data obtained in the 10 tests.

Inhibition tests of methane production with wastewater. For these tests, four samples of wastewater were collected from a brewery that uses the chain lubricant of the mentioned type. The samples were taken at the exit of the equalization tank. The concentration of volatile fatty acids (VFA), the concentration of organic matter (COD) and the pH were determined for each sample. As the samples had a pH lower than 7, and it was necessary to avoid the inhibitory effect by this factor, the samples were neutralized by adding sodium bicarbonate. Inhibition tests were carried out with the undiluted waste water. Each test included 4 replicates per sample, and the aforementioned controls. Information processing. To calculate the IC_{50} values and the associated 95% confidence interval in the PCP and lubricant tests, the parametric Probit statistical method was used (USEPA 1991).

The results obtained with the PCP, adjusted to the linear model (Probit), were used to construct the control chart. The elaboration of this chart was carried out by graphing the obtained IC50 values as a function of the tests carried out over time. Based on this graph, the mean IC50 value and its respective standard deviation (S) were calculated. With these values, the interval in which the IC50 should be maintained (Mean ± 2S) was determined to guarantee precision within a 95% confidence limit.

Likewise, the value of the concentration of PCP and lubricant that does not produce an observable effect in the acetoclastic methanogenic population (NOEC) was calculated. The value was obtained from the equation obtained in the regression analysis of the concentration / response graph.

To define whether the percentage of inhibition found in the wastewater was significant or not, the Pass / Fail procedure proposed by USEPA (1991) for tests with a single concentration was followed. This procedure requires prior evaluation of the normality and homogeneity of variances of the data from the control group and those from the treatment. Using the Wilcoxon hypothesis test, it is compared whether or not there is a significant difference between the treatment and the control.

RESULTS AND DISCUSSION

Anaerobic mud characteristics. Table 1 shows the concentration of solids presented by the selected mud. As can be seen, the SSV / SST ratio indicated a high biomass concentration indirectly measured by the concentration of volatile suspended solids. These values were higher than those reported by Espitia (1999) for five sludge from other brewery reactors. Taking into account the recommended values for tests of methanogenic activity, the values found had the appropriate proportion of a sludge, to be used as inoculum. These parameters were repeatedly evaluated during a year, and the results allowed to verify that under conditions from storage the solids concentration did not vary (% $C.V_{SSV}$ = 1%). As it was expected that bacterial activity would not decrease either, monthly measurements of methanogenic activity were made, which are presented in figure 1. As can be seen, the activity remains relatively constant with an average of 0.87 ± 0.04 g $DQO_{CH}4$ / gSSV * d and a coefficient of variation of 4.6%.

Table 1. Concentration of solids in anaerobic sludge.

Parameter	Concentration (g / l)
Total Solids (STT)	99.33
Total Volatile Solids (STV)	61.3
Total Suspended Solids (SST)	51.85
Volatile Suspended Solids (SSV)	32.14
SSV / SST Ratio	0.62

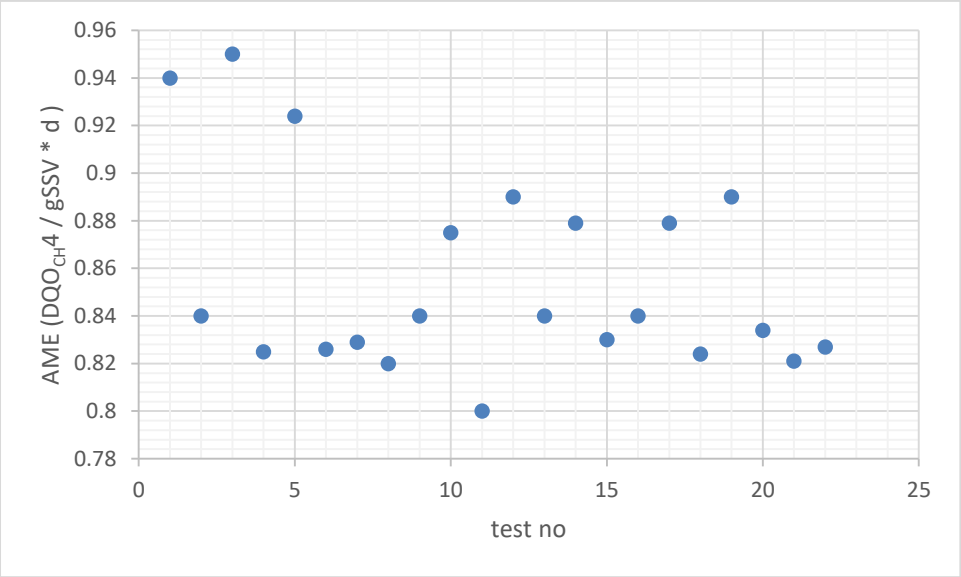

Figure 1. Variation over time of the methanogenic activity of anaerobic sludge

The analysis of the microbial composition showed a structure in which hydrogen-using methanogens (BMH) and acetate-reducing sulfate (BSA) predominated, and to a lesser extent, glucose and lactate fermentative bacteria (Figure 2). This phenomenon is consistent with the type of substrates present in brewery wastewater, used as electron donors (Wu et al., 1991). The predominance of hydrogenophilic methanogenic archaea can be explained by the need to maintain a partial pressure of hydrogen at a level that allows the syntrophic degradation of ethanol and propionate (Wu et al., 1991). The high count of sulfator-reducing bacteria in systems with low sulfate content is related to the ability of this group to grow using acetogenic metabolism, through incomplete oxidation of ethanol (Scholten, 1999). In addition, as the sludge came from a separate phase reactor, in which the initial degradation stages (fermentation and acidogenesis) are carried out in an acidic tank, it is to be expected that the glucose and lactate concentration in the methanogenic reactor is too low to support a high number of fermentative bacteria. Inhibition of methane production with pentachlorophenol. The inhibitory effect of PCP on methane production is presented

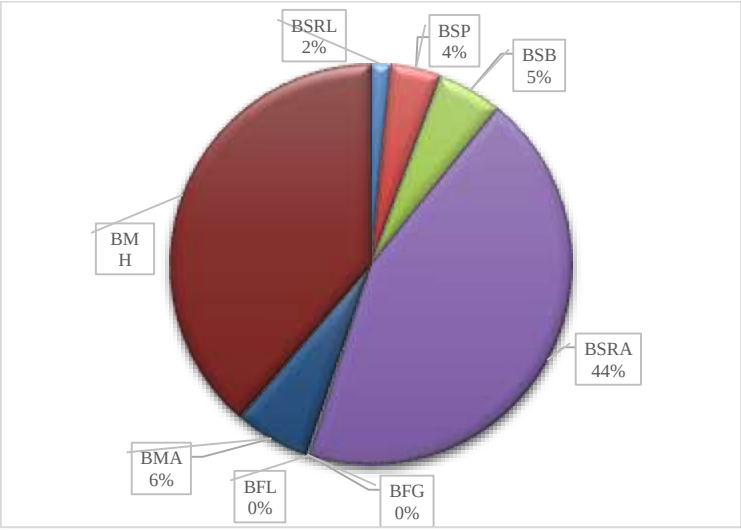

Figure 2. Percentage distribution of the microbial populations present in the anaerobic sludge. Glucose and lactate fermentative bacteria (BFG, BFL), sulfate-reducing acetate and lactate bacteria (BSRA, BSRL), propionate and butyrate syntrophic bacteria (BSP, BSB) and methanogenic hydrogen and acetate archaea (BMH, BMA).

The average value of the C_{50} at 48 hours for the PCP was 0.043 mM with a coefficient of variation of 17.85%. Although it would seem that the variability between the different tests is high when compared with a chemical determination, the value found is within the acceptable limits for biological tests, which can reach 25% (Bertoletti, 1992). In these bioassays, not only the variability of an organism is measured, but also that of the bacterial consortium, which, due to the amount of factors that can affect it, are an important source of variation.

The value found for the IC_{50} of the PCP was similar (0.03mM) to that reported by Sierra and Lettinga (1991), and is located within the confidence interval (95%) obtained for the $C_{/50}$ = 0.043 mM found in this work [0.028-0.058]. The results show not only the reproducibility of the tests carried out, but also the possibility of incorporating this methodology to study the toxic effect of different substances on anaerobic populations.

Table 2 shows that low concentrations (0.043 mM) of PCP are capable of inhibiting methane production by 50%. However, its activity can vary with different types of bacteria. Ruckdeschel et al. (1987) evaluated the action of PCP and its metabolites with 30 different bacterial strains. The results showed that, for the species of the genera Clostridium, Mycobacterium and Streptomyces, the Minimum Concentration Inhibits the concentration-response curve (figure 3). As can be seen, inhibition increases proportionally with increasing concentration. Regression analysis revealed that the observed behavior conformed to a linear model described by the equation: Y (% /) = 1398.6x (mM PCP) - 6.381. Based on this equation, the calculated value for NOEC was 0.003 mM.

Inhibition of methane production with the lubricant. As with PCP, the lubricant showed an inhibitory effect proportional to the concentration (figure 4). The maximum methane production rate decreases with increasing lubricant concentration, and the behavior fits a linear model described by the equation: Y (ml CH4 / h) = -9.0824x (% v / v lubricant) + 2.0408 (r^2 = 0.9579). By writing the equation in terms of the variables, the model allows predicting that the inhibition of the lubricant on the methanogenic population would be: TMPM (ml CH_4 / h) = TMPMc (ml CH4 / h) + □ * [lubricant% v / v], where the TMPMc value corresponds to the maximum rate of methane production in the negative control (1.96) and □ to the slope of the graph, a measure of the inhibitory potential of the lubricant, which for this case was -8.8.

Table 2. CI values, obtained for pentachlorophenol in the different methane production inhibition tests.

Test	CI_{50} mM 95% confidence	interval
1	0.052	0.044-0.067
2	0.035	0.032-0.039
3	0.043	0.039-0.050
4	0.034	0.030-0.039
5	0.049	0.044-0.057
6	0.036	0.029-0.050
7	0.048	0.043-0.055
	S=0.0075	% C.V = 17:58

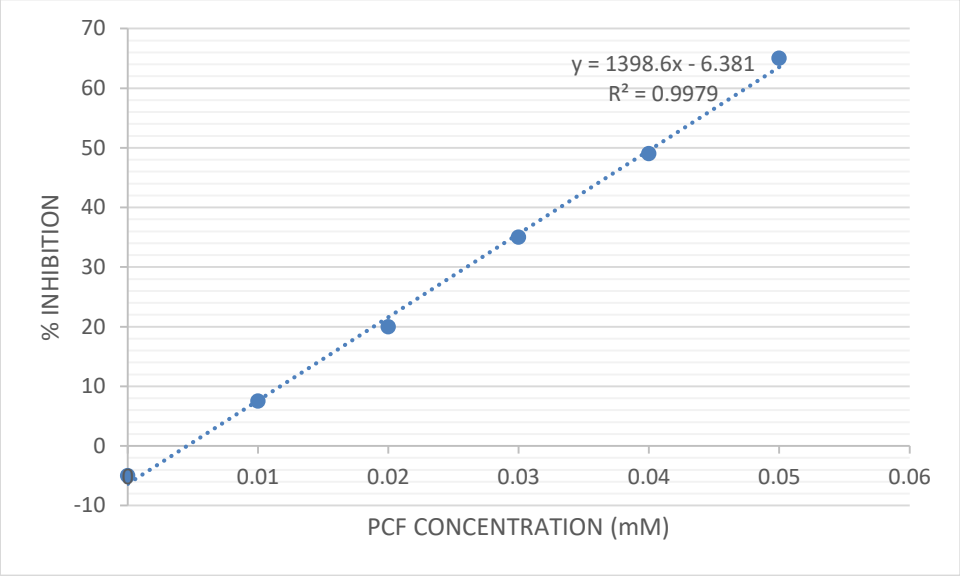

Figure 3. Concentration-response curve for pentachlorophenol

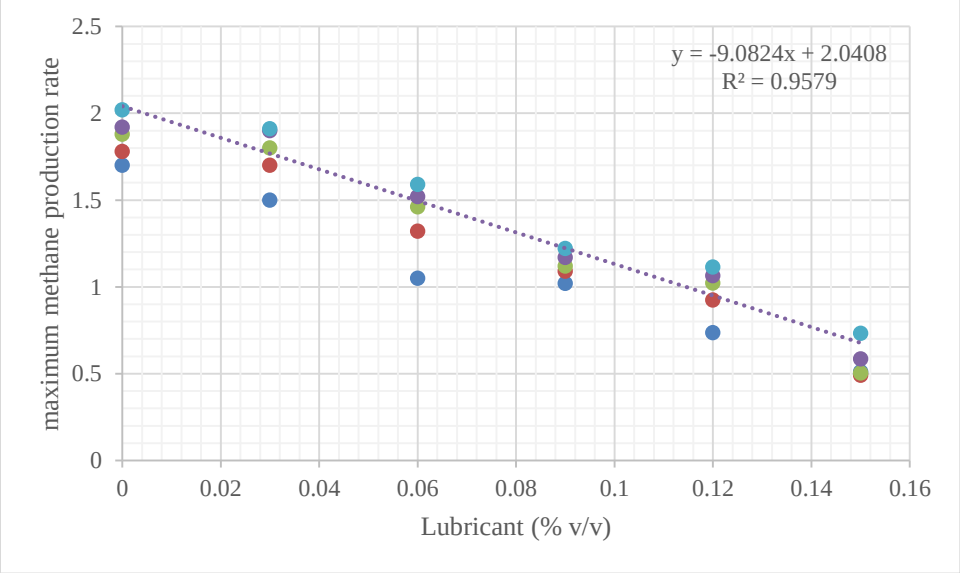

Figure 4. Maximum methane production rate as a function of lubricant concentration.

Studies carried out on the effect of anionic surfactants of the linear alkyl-benzene-sulphonated (LAS) type, in acetoclastic acidogenic and methanogenic populations of thermophilic sludge (García-Morales et al., 2001), showed that there is a linear relationship between the rate of methane production and surfactant concentration, with a p-value of -0.033, lower than that obtained in this work. This would indicate that the cationic type lubricant could have a much higher toxic potential than anionic surfactants. Therefore, the observed toxicity would not only be the result of the lubricant's surfactant activity, but also of other chemical properties that could contribute to generating the observed toxic effect.

Although the microbicidal potential of cationic surfactants has been known since 1935 (Schuartz and Perry, 1949), the mechanism by which these compounds exert their toxic activity is not clear. In general, this activity is related to the chemical structure of cationic surfactants, and the presence of hydrophobic groups, capable of reducing surface tension. These compounds are adsorbed to the surface of bacteria, preventing the interaction of cells with the surrounding environment, which leads to the interruption of many metabolic functions. However, it is not ruled out that other factors contribute to the toxicity of these compounds.

Based on the concentration-response curve (figure 5), the equation was obtained: / (% /) = 632.92x (% v / v lubricant) - 3.6889, from which the NOEC value was calculated (0.009% v / v). The average IC_{50}-48h was 0.11% v/v. The coefficient of variation found was 15.4% of the same order as that obtained in the trials with PCP. The IC_{50} value was 10 times higher than that reported by Nagel et al. (1999) for a non-ionic synthetic lubricant (0.018% v / v).

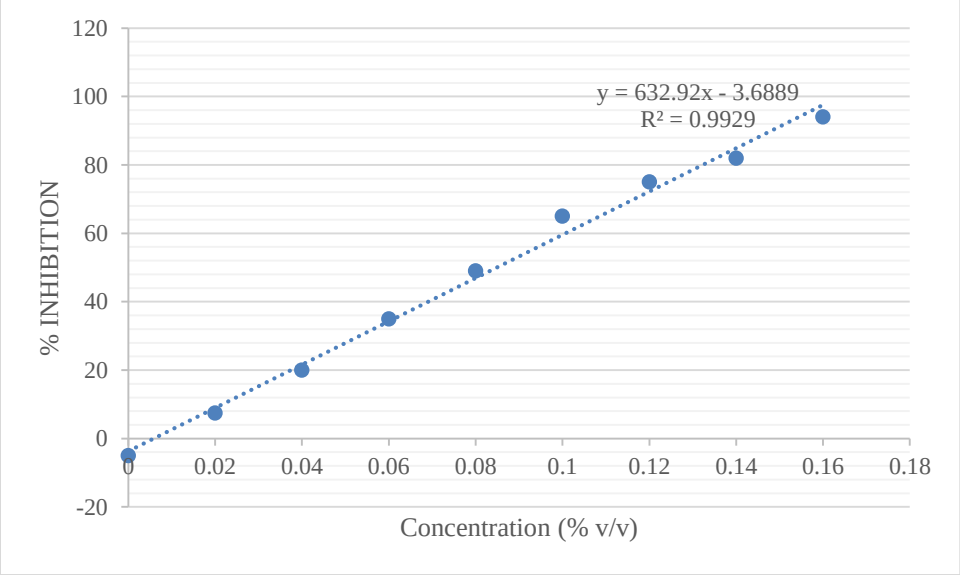

Figure 5. Concentration-response curve for the lubricant.

The results suggest that, under the test conditions, any lubricant concentration above 0.009% v / v (NOEC) could exert an adverse effect on the acetoclastic methanogenic population. However, it is necessary to make experimental determinations with concentrations between 0.009 and 0.03% v / v that allow to confirm or reject this extrapolation. In addition, in real operating conditions, the lubricant is not only highly diluted, but there are also factors such as pH, concentration of a large amount of organic matter and the presence of other chemical compounds, which can affect toxic activity. of pure lubricant.

Inhibition of methane production with wastewater. The toxic effect measured in the four residual water samples is recorded in figure 6. It shows the value of the maximum methane production rate obtained with each of the replicas as well as in those of the negative control. . The lowest values were observed in sample 1 (1.7-1.81 ml CH4 / h). The result of the Pass / Fail test procedure proposed by USEPA for single-concentration toxicity tests showed that the inhibitory effect was significant for samples 1, 2 and 4 (⍰ = 0.05).

Although the inhibition values found are relatively low, 11.4%, 7.7% and 8.1% for samples 1, 2 and 4, respectively, this fact can be considered as an alert about the possible presence of substances that can trigger events of toxicity.

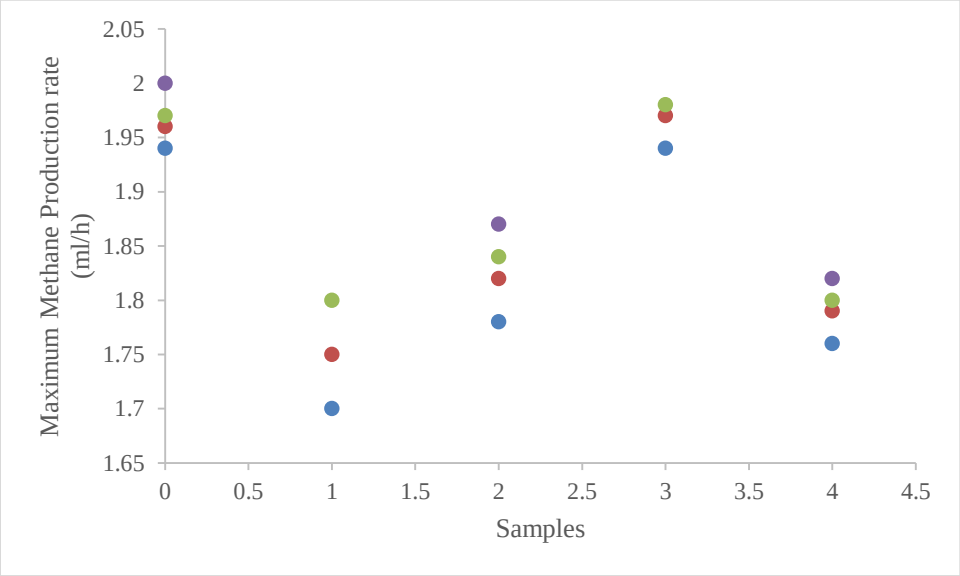

Figure 6. Maximum rate of methane production in the negative control and in the residual water samples.

In order to estimate the concentration of the lubricant in the wastewater, given the difficulty in quantifying it by chemical analysis, a mass balance was carried out. For this, the hourly data of the inflow to the treatment plant for a period of 15 days (García, 2003) was taken as a basis on the average daily consumption of lubricant (65 kg / d), and it was assumed that the lubricant it did not biodegrade. The results obtained show that the estimated lubricant concentration in the wastewater would be between 0.00061 and 0.0017% v / v. This value is well below the calculated value of NOEC (0.009% v / v). For this reason, it was considered that the toxicity detected could not be associated with the lubricant, since the estimated concentration would not be sufficient to cause the observed inhibitory effect.

Therefore, it is clear that, from the results obtained with the mass balance, an association cannot be made between the observed inhibition and the presence of the lubricant in the wastewater. Therefore, to make this type of association it will be necessary to carry out a chemical identification procedure coupled with fractionation techniques. Despite the above, the results indicate that the methodology used allows detecting toxic events, which can contribute to improving the operation of these reactors.

CONCLUSIONS

The methane production inhibition tests allowed establishing toxicity parameters such as IC_{50}.Inhibition assays of methane production with POP showed that the test is reproducible, and that concentrations above 0.01 mM have a strong inhibitory effect on the methanogenic population.The tests with the pure lubricant made it possible to establish that a concentration of 0.03 mM or higher exerts a strong inhibitory effect on the production of methane. Although the mass balance could not establish an association between the observed toxic effect and the estimated lubricant concentration in the wastewater, it is clear that temporary toxicity events can occur, whether generated by the lubricants or other compounds that can inhibit methane production.

References

[1]. Adams, R., Domínguez, V., García, L. 1999. Bioremediation potential of soil and water impacted by oil in the Mexican tropics. Terra 17 (2): 159-174.

[2]. Ambrosoli, R., Petruzzelli, L., Minati, J., Marsan, F. 2005. Anaerobic PAH degradation in soil by a mixed bacterial consortium under denitrifying conditions. ScienceDirect 60 (9): 1231-1236.

[3]. Araújo, I., Angulo, N., Cárdenas, C., Méndez, M., Morante, M., Machado, M. 2005. Soil bioremediation with bacterial consortium, composting and fertilization. Biological. INCI 38 (3): 186-202.

[4]. Araújo, I., Mantilla, M., Cardenas, C., Herrera, L., Angulo, M., Murillo, G. 2006, Stabilized sludge and bacterial strains in the Bioremediation of soils contaminated with hydrocarbons, Interciencia 31 (004) : 268-275.

[5]. Arroyo, M., Quesada, M., Quesada, R. 2008. Application of bioremediation systems for soils and water polluted by hydrocarbons. Geocisa. Div. Soil Environmental Protection: 297-305. Available at http://aguas.igme.es/igme/publica/pdflib15/028.pdf

[6]. Atlas, M. 1981. Microbial Degradation of Petroleum Hydrocarbons: an Environmental Perspective. Microbiological Reviews 45 (1): 180-209.

[7]. Benavides, L. J., Quintero, G., Ostos, O. 2006. Isolation and identification of ten denitrifying bacteria from agricultural soil contaminated with nitrogen fertilizers from an onion-producing farm. Nova - Scientific Publication 4 (006): 50-54.

[8]. Benavides, L. J., Quintero, G, Guevara, V. A., Jaimes, D. C. Bioremediation of soils contaminated with petroleum-derived hydrocarbons. Colombia. Not going. 4 (5): 1-116.

[9]. Boogaard, P., Sittert, N. 1994. Exposure to polycyclic aromatic hydrocarbons inpetrochemical industries by measurement of urinary 1 -hydroxypyrene. Occupational and Environmental Medicine 51: 250-258.

[10]. Bregnard, T., Haner, A., Hohener, P., Zeyer, J. 1997. Anaerobic Degradation of Pristane in Nitrate-Reducing Microcosms and Enrichment Cultures. Appl Environ Microbiol 63 (5): 2077-2081.

[11]. Brissio, P. A. 2005. Preliminary evaluation of the state of contamination in soils in the province of Neuquén where hydrocarbon exploitation activities are carried out. National University of Comahue. Neuquén, Argentina.

[12]. Cardenas, C., Araújo, I., Bohórquez, M., Gómez, K., Angulo, N., Gómez, A. 2004. Influence of fertilization in the Bioremediation of soils contaminated with hydrocarbons using stabilized residual sludge. Venezuela. Available at http://www.ingenieroambiental.com/4014/angulo.pdf

[13]. Cañas, J., Jerez, T. 2003. Isolation and identification of fungi with hydrocarbon degrading capacity. Phase III bioremediation. University of Santander, Bucaramanga.

[14]. Cheung, P., Kinkle, B. 2001. Mycobacterium diversity and pyrene mineralization in petroleum-contaminated soils. Applied and Environmental Microbiology 67 (5): 2222-2229.

[15]. Basel Convention on the Control of the Transboundary Movement of Hazardous Wastes and their Disposal. 2000. Methodology guide for conducting national inventories of hazardous waste within the framework of the Basel convention. First version, Basel Convention series / SBC 99/009 (S), Geneva.

[16]. Corona, L., Iturbe, R. 2004. Natural attenuation in soils contaminated with hydrocarbons. Engineering Research and Technology 2: 119-126.

[17]. Demaneche, S., Kay, E., Gourbiere, F., Simonet, P. 2001. Natural transformation of Pseudomonas fluorescens and Agrobacterium tumefaciens in soil. Applied and Environmental Microbiology 67 (6): 2617-2621.

[18]. Decora, A. Kerrt, R. 1979. Processing Use, and Characterization of Shale Oil Products. Environmental Health Perspectives 30: 217-223.

[19]. Fonseca, A., Vargas, F. 2006. Evaluation of microbial consortia with degrading capacity of hydrocarbon residues and their application in bioremediation of soils contaminated with used lubricating oils. University of Santander, Bucaramanga.

[20]. Findley, J., Appleman. M., Yen, T. 1974. Degradation Of Oil Shale By Sulfur-Oxidizing Bacteria. Applied Microbiology 28 (3): 460-464.

[21]. Kasai, Y., Takahata, Y., Hoaki, T., Watanabe, K. 2005. Physiological and molecular characterization of a microbial community established in unsaturated, petroleum-contaminated soil. InterScience 7 (6): 806-18.

[22]. Koren, O; Knezevic V; Ron E, Rosenberg E. 2003. Petroleum Pollution Bioremediation Using Water-Insoluble Uric Acid as the Nitrogen Source. Applied and Environmental Microbiology 69 (10): 6337-6339.

[23]. Leary, J., Biemann, K., Lafleur, A., Kruzel, T., Prado, G., Longwell, J., Peterst, W. 1987. Chemical and Toxicological Characterization of Residential Oil Burner Emissions: I. Yields and Chemical Characterization of Extractables from Combustion of No. 2 Fuel Oil at Different Bacharach Smoke Numbers and Firing Cycles. Environmental Health Perspectives 73: 223-234.

[24]. Littell, R. C., Milliken, G. A., Stroup, W. W., Wolfinger, R. D., Schabenberger, O. 2006. SAS® for Mixed Models, Second Edition. Cary, NC: SAS Institute Inc.

[25]. Lopera, E., Aguirre, J. 2006. Purification of insulating oils contaminated with Polychlorinated Biphenyls (PCBs). Dyna 73 (150): 75-88.

[26]. Martín, C., Aldo González, A., Blanco, M. 2004. Biological treatments of contaminated soils: contamination by hydrocarbons. Mushroom applications in bio-recovery treatments. Ibero-American Journal Microbiology 21: 103-120.

[27]. Mashreghi, M., Marialigeti, K. 2005. Characterization of Bacteria Degrading Petroleum Derivatives Isolated from Contaminated Soil and Water. Journal of Sciences 16 (4): 317-320.

[28]. Menezes, F., Oliveira, F., Okeke, B., Frankenberger, W. 2003. Bioremediation of soil contaminated by diesel oil. Brazilian Journal of Microbiology 34 (1): 65-68.

[29]. Narváez, M., Martínez, M. 2008. Selection of bacteria with hydrocarbon degrading capacity, isolated from sediments of the Colombian Caribbean. Bol Invest Mar Cost Colombia 37 (1): 63-77.

[30]. Noren, K., Meironyté, D. 2000. Compounds in Human Environments. Environmental Health Perspectives 59: 145-158.

[31]. Ochoa, E. 2003. Bioremediation phase I, Universidad de Santander, Bucaramanga.

[32]. Plaza, G., Otero, M., Torres, N., Velásquez, M., Corbalán, E., Rodríguez, T. 2001. Bioremediation in soils contaminated with hydrocarbons. Advances in Renewable Energies and the Environment 5: 1-5.

[33]. Perera, F. 1981. Carcinogenicity of Airborne Fine Particulate Benzo (a) pyrene: An Appraisal of the Evidence and the Need for Control Environmental Health Perspectives 42: 163-185.

[34]. Quintero, A., Quintana, A. 2005. Phase IV bioremediation. University of Santander, Bucaramanga.

[35]. Ran, Xl., Obbard, J. 2004. Biodegradation of Polycyclic Aromatic Hydrocarbons in Oil-Contaminated Beach Sediments Treated with Nutrient Amendments. Published in J. Environmental 33: 861-867.

[36]. Rockne, K., Chee-Sanford, J., Sanford, R., Brian, P., James, T., Staleyand, S. 2000. Anaerobic naphthalene degradation by microbial pure cultures under nitrate-reducing conditions. Applied and Environmental Microbiology 66 (4): 1595-1601.

[37]. Steffen, K., Hatakka, A., Hofrichter, M. 2003. Degradation of Benzo [a] pyrene by the Litter-Decomposing Basidiomycete Stropharia coronilla: Role of Manganese Peroxidase. Applied and Environmental Microbiology 69 (7): 3957-3964.

[38]. Vallejo, G., Arnau, J., Bono, R., Fernández, G. P., Tuero, H. E. 2008 Construction of hierarchical models in applied contexts. Psicothema 20 (004): 830-838.

[39]. Vargas, P., Cuéllar, R., Dussán, J. 2004. Bioremediation of petroleum residues. Uniandine Scientific Notes 4: 43-49.

[40]. Lamma, O.A. and Swamy, A.V.V.S., 2018. Assessment of Ground Water Quality at Selected Industrial Areas of Guntur, AP, India. Int. J. Pure App. Biosci, 6(1), pp.452-460.

[41]. Lamma OA, Swamy AV, Subhashini V.,2018 . GROUND WATER QUALITY IN THE VICINITY OF INDUSTRIAL LOCATIONS IN GUNTUR, AP, INDIA

[42]. Lamma, Osama Asanousi. "Groundwater Problems Caused By Irrigation with Sewage Effluent." International Journal for Research in Applied Sciences and Biotechnology 8, no. 3 (2021): 64-70

[43]. Lamma, O., & Swamy, A. V. V. S. (2015). E-waste, and its future challenges in India. Int J Multidiscip Adv Res Trends, 2(I), 12-24.

[44]. Mohammad, M. J., Krishna, P. V., Lamma, O. A., & Khan, S. (2015). Analysis of water quality using limnological studies of Wyra reservoir, Khammam District, Telangana, India. Int. J. Curr. Microbiol. App. Sci, 4(2), 880-895.

[45]. Lamma, O. A., & Moftah, M. A. (2016). Effect of vermicompost on antioxidant levels in Andrographis paniculata. International Journal of Applied and Pure Science and Agriculture, 2(3), 160-164.

[46]. Outhman, A. M., & Lamma, O. A. (2020). Investigate the contamination of tissue paper with heavy metals in the local market. IJCS, 8(1), 1264-1268.

[47]. Lamma, O. A. (2021). Groundwater Problems Caused By Irrigation with Sewage Effluent. International Journal for Research in Applied Sciences and Biotechnology, 8(3), 64-70.