

## APPLICATION OF FLUORESCENT *IN SITU* HYBRIDIZATION IN THE EVALUATION OF THE BIOCIDES USED IN THE OIL INDUSTRY

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Microbiologically influenced corrosion (MIC) and souring of oilfield reservoirs as result of the presence of sulfate-reducing bacteria (SRB) are of great concern in the oil industry. Considering the limitations of conventional culture-based methods for studying SRB, techniques involving the direct analysis of the microbial population from their genetic material are getting more attention. One of them is Fluorescent *in situ* Hybridization (FISH), which uses fluorescently labeled oligonucleotide probes that hybridizes specifically to its complementary 16S rRNA target sequence within the intact cell. Using FISH, the abundance of the detected microorganisms can be determined by counting the cells stained with a general DNA-binding dye and the cells hybridized with a specific probe. To control bacterial populations, biocides are commonly applied to injection waters and production facilities. The aim of this work was to evaluate the possibility of using FISH to help in the selection of an appropriate biocide for the water treatment plant of an oil secondary recovery plant (OSRP). Three commercial biocides based on THPS and quaternary ammonium salts were used in the assay in a concentration of 200mg/l. Three replicates of each biocide in each concentration were done. The following probes (5P end-labeled with Cy3) were used: Eub338; Non338 and SRB385. The fluorescence was detected with a Leica microscope, analyzing 15 to 20 images per sample. The inhibition effect of the biocides was tested in OSRP water filtered inoculated with a microbial culture in PostageB medium, obtained from the same water. The inhibitory effect was determined by counting the cells hybridized with the Eub338 and SRB385 probes. The percentage of hybridized vs DAPI-stained cells, RS%, was calculated. The RS% values for the Eub338 probe obtained after 4h of incubation at 60°C (water treatment plant condition) in the presence of biocides B1 and B3 were higher than those obtained with the control (without biocide). This behavior suggested a metabolic activation, associated with a high content of RNA in the cells. It could be related with the mode of action of the chemicals or the application of a sub lethal dose. The RS% values obtained with B2 were no significantly different from those of the control, associated with no inhibitory effect. The RS% values obtained after 4h of incubation for SRB population showed that none of the tested biocides produced metabolic activation. B1 and B3 biocidal effects were not different from that observed in the control. Instead, the lower RS% values obtained with B2 were associated with a significant inhibitory effect on SRB population, showing that the SRB population was more sensitive to B2 effect under the tested condition. These results suggested that FISH could be helpful in the screening of biocides, being a responsive and suitable test to detected inhibitory concentrations.

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## CHEMICAL AND MICROBIOLOGICAL CHARACTERIZATION OF EFFLUENTS IN DAIRY FARMS OF RIO PRIMERO DEPARTMENT (CÓRDOBA, ARGENTINA)

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The main problem of milk production is the generation of effluents containing excreta, organic matter (OM), nutrients, etc. If effluents are not adequately managed affect the quality of water, soils and human health. Therefore the utilization of the effluents for irrigation of crops and fodder after of its treatment with system of 2 or 3 lagoons is a good option to minimize effluents impact. There is little local information on the quality of livestock effluents and the impact of its discharge. The objective was to evaluate the effluents quality in order to determine the efficient of the treatments used. It is worked in three dairy farms of Río Primero Department, Córdoba. In the first (DF1) and second dairy farm (DF2) the water used is deposited in one lagoon that when is filled the content is discharged to the field. The third dairy farm (DF3) presented two lagoons: one for the water of the first wash (T3-FW) and other for the second wash (T3-SW). Two water samples were taken in each lagoon: one near the effluent input (I) and other in the farthest area (F). The samples were collected in sterile receptacles and stored refrigerated until analyses. In each sample the following parameters were determined: a) pH, b) total organic matter (TOM), c) mesophilic bacteria, d) total and fecal coliforms and e) *Escherichia coli*. The obtained data were compared with the allowable limits for the use of effluents for irrigations (Environmental Protection Agency of EEUU-EPA) and effluent discharge to surface water courses (Subsecretaría de Recursos Hídricos de Córdoba - SRH). The DF1 sample showed an alkaline pH, while the other samples presented pH near the neutrality, except for DF3-FL that showed an acid pH. The TOM was higher in the samples near the effluent input, whereas the lower values were obtained in DF2-F. All samples presented an elevated number of mesophilic bacteria, total and fecal coliforms and *E. coli*. No effluent met the allowed values ( $\leq 1000$  fecal coliforms/100 mL) according EPA and SRH. The fecal coliforms exceeded the allowable limits, while pH values met the norms, except for T3-FW (I). Our results