Journal of Petroleum Science and Technology

Crude Glycerol as a Substrate for Sulfate-reducing Bacteria from a Mature Oil Field and its Potential Impact on Souring

Sidnei Cerqueira dos Santos[1]*, Martin Gerald Liebensteiner[2], Leila Cristiane Silva das Virgens de Souza[1], Catia Larissa Santos Ramos[1], Luiz Lázaro Franco Batista[1], Josilene Borges Torres Lima Matos[1], Cristina Maria Quintella[3], and Paulo Fernando de Almeida[1]

[1] Laboratory of Biotechnology and Ecology of Microorganisms, Department of Bio-Interaction Sciences, Federal University of Bahia, Brazil

[2] Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands

[3] Laboratory of Kinetic and Molecular Dynamic, Institute of Chemistry, Federal University of Bahia, Salvador, Brazil

ABSTRACT

Crude glycerol (CG) is an abundantly available and cheap by-product from biodiesel production. Value-added applications for CG are highly wanted by industry and several processes such as the use of CG for enhanced oil recovery have been proposed. The aim of this study was to evaluate the sulfide production of sulfate-reducing bacteria (SRB) indigenous to oil reservoirs using CG as substrate. The samples of CG were obtained from a biodiesel production plant, processing castor beans, soybeans, cotton, and waste oils and fats. Growth tests were performed in Postgate medium, with different types and concentrations of CG, and a mixed inoculum of SRB isolated from the produced water of a mature oil well of Bahia (Brazil). The experiment was monitored by measuring the concentration of sulfide using a colorimetric method. The results showed that SRB grew and produced more than 250 ppm sulfide at CG concentrations of 2%. However, at CG concentrations of 3% or higher, the biogenic production of sulfide was reduced. The study demonstrates that CG will likely stimulate SRB in oil fields, whenever CG is present at lower concentrations. Maintaining CG concentrations inhibitive to SRB will not certainly be achievable throughout oil reservoirs. Dosing CG to oil fields may lead to problems associated with souring in longer terms. The utilization of CG by SRB could in turn be interesting for other biotechnological processes, e.g. metal recovery processes based on precipitation with biologically formed sulfide.

Keywords: Souring, Sulfate-reducing Bacteria, Crude Glycerol, Inhibition

INTRODUCTION

Crude glycerol (CG) is the main by-product of biodiesel production. It accounts for up to 10% of the product and contains between 40 and 90% pure glycerol [1,2]. The growth of biodiesel production has resulted in an excess of glycerol and consequently in a decline of the market price for glycerol [3,4]. This glycerol is considered as waste due to the presence of impurities such as alcohol, salts, and heavy metals,

***Corresponding author**
Sidnei Cerqueira dos Santos
Email: sidnei.cerqueiradossantos@gmail.com
Tel: +55 71 3283 6711
Fax: +55 71 3235 5166

Article history
Received: December 22, 2013
Received in revised form: May 31, 2014
Accepted: June 25, 2014
Available online: February 20, 2015

which make it unsuitable for direct industrial use and its purification has a higher cost than obtaining it from other sources [5]. This scenario indicates that the commercial viability of biodiesel calls for new and sustainable solutions for the consumption of crude glycerol [6].

New strategies are being developed with the aim to increase the economic value of this by-product [4,6] such as the use of CG as fluid for oil recovery in the micro-reservoir cell. The latter could achieve an oil recovery factor of 80% and can thus be regarded as a promising method for the petroleum industry [7]. However, there is still limited knowledge about the microorganisms utilizing the injected CG and what products are formed during the process. The injection of crude glycerol in oil wells could possibly stimulate the growth and sulfide production of SRB, which are widely distributed in anoxic environments like oil reservoirs [8,9]. Microbial sulfide production in oil reservoirs has been a major problem in the petroleum industry (called souring), been lowering the productivity and quality of produced hydrocarbons, been increasing corrosion, and causing safety, health, and environmental problems [10,11]. A potentially stimulating effect of CG on SRB which are indigenous to oil reservoirs would therefore intensify the problem of souring and thus have a negative impact on its usability for enhanced oil recovery purposes.

The aim of this study was to evaluate the use of crude glycerol by oil reservoir inhabiting sulfate-reducing bacteria. A mixed culture of sulfate-reducing bacteria isolated from a mature oil field (Bahia, Brazil) was incubated with different sorts of crude glycerol from biodiesel production as the only carbon and energy source and the biological formation of sulfide was followed over time.

MATERIALS AND METHODS

Produced Water (PW)

The samples of the produced water were collected from a mature oil field of Bahia (Brazil) in 2009. The reservoir temperature was 44 °C, the initial pressure was 55 bar [12] and the salt concentration of the collected samples was 3.5%. The pH and sulfide concentration of the produced water were analyzed after centrifugation.

Quantification and Selection of SRB

The quantification of sulfate-reducing bacteria from the produced water samples was done by the most probable number (MPN) method, according to the American Society for Testing and Material [13]. MPN estimates were calculated from statistical tables [14]. Serial dilutions were performed in modified Postgate E medium, containing the following (g/l): NaCl, 35.0; $C_6H_5Na_3O_7$, 6.38; $MgCl_2.6H_2O$, 1.83; KH_2PO_4, 0.5; NH_4Cl, 1.0; Na_2SO_4, 1.0; $CaCl_2$, 1.0; yeast extract, 1.0; ascorbic acid, 0.1; sodium thioglycolate, 0.01; $FeSO_4.7H_2O$, 0.5; agar, 1.5; sodium lactate, 1.75 ml (60%, w/w); resazurin, 2.0 ml (0.025%, w/v). The pH was adjusted to 7.5-8.0 using HCl or NaOH and the medium was sterilized at 121 °C for 20 min. The samples were incubated in an anaerobic chamber (Bactron VI, Shellab, Sheldon Manufacturing Inc.) at 38 °C for 21 days. The growth of sulfate-reducing bacteria was indirectly followed by the formation of iron sulfides (appearing black), only occurring if sulfate reduction takes place.

Aliquots of 0.1 ml of positive MPN cultures were transferred to solid Postgate medium (agar, 15 g/l), and streaked using the spread plate method [15]. The plates were incubated in the anaerobic chamber at 38 °C. The selection of SRB was made based on macroscopic features and the formation of black precipitates in the colonies. A mixed culture of sulfate-reducing bacteria, called APB55, was composed by the selection of five different pure colonies. The morphological characteristics of the particular strains in the mixed culture were confirmed by microscopy.

Identification of Sulfate-reducing Bacteria (SRB)

Fluorescence in situ hybridization (FISH) technique was used to identify the presence of sulfate-reducing bacteria in APB55, according to standard procedures [16]. We used the indocarbocyanine (Cy3)-labeled 16S rRNA oligonucleotide probes SRB-385 (5'-CGGCGTCGCTGCGTCAGG-3') for the SRB group of the δ-Proteobacteria [17], DSV-698 (5'-GTTCCTCCAGATATCTACGG-3') for the Desulfovibrionaceae family [18], and SRB-129 (5'-CAGGCTTGAAGGCAGATT-3') for the Desulfobacter genus [19]. The total amount of cells was visualized by using DAPI (4',6-diamino-2-phenyl indole), a DNA-binding stain. Fluorescence microscopy equipped with two sets of filters (U-MWU2-330/420nm and U-MSWG2-480/590nm) was used to detect DAPI and Cy-3 stained cells respectively [20]. The slides were analyzed using a microscope (Olympus®, BX51) coupled to a camera (Olympus®, Q-Color). Image analyses were carried out using the software Image Pro-Plus 5.1.

Crude Glycerol (CG)

The samples of crude glycerol were collected in the Petrobras Biofuel plant, situated in Candeias (Bahia, Brazil). The CG samples differed regarding the raw materials used for biodiesel production, namely oil from castor beans, soybeans, cotton, and waste oils and fats (WOF) as raw materials. The CG samples were sterilized in an oven at 170 °C for 1 hour [21]. The chemical composition of the crude glycerol samples was determined according to the analytical methods mentioned below.

Evaluation of Sulfide Production

The assay of biogenic sulfide production was carried out in test tubes containing 20 ml of modified Postgate E medium containing crude glycerol as the sole carbon and energy source (leaving out sodium citrate and sodium lactate). Four different samples of CG from biodiesel production were used at concentrations of 1-5% (w/v). Sterilized commercial glycerol (99.5%, Merck) was used as a control. Inocula of 10^3-10^5 cells/ml of APB55 were added to the test tubes. Epifluorescent microscopy counting acridine orange (3,6-bis dimethylamino acridinium chloride) stained cells was carried out to standardize the inocula of APB55 [22]. Before staining, the culture was washed with Tween 80 (0.1%) and centrifuged at 7500 g for 10 minutes. The slides were analyzed with an Olympus® BX51 microscope. The test tubes were incubated in an anaerobic chamber at 38 °C for 96 hours. Aliquots of the incubated tubes were collected to analyze the sulfide production. The tests were performed with duplicates. The sulfide production was expressed by using the arithmetic mean and the range of duplicates.

Analytical Methods

For the pH measurements a pH-Fix 0-14 tape (Macherey-Nagel) was used. Sulfide was determined with the N,N-dimetil-p-fenilenediamina method [23]. The sulfide content of the cultures was measured at 660 nm on a spectrophotometer (PerkinElmer, Vitor 1420). The standard curve was prepared from the dilution of a standard solution of sodium sulfide and results were expressed in parts per million (ppm). The glycerol concentration of the CG samples was determined by an enzymatic assay measuring triglycerides (Doles), which is also sensitive to glycerol, and the absorbance was measured at 510 nm. Aldehydes were quantified by extracting them with acetonitrile (Merck) followed by a derivatization step with an acid solution of 2,4-dinitrophenylhydrazine. The carbonyl hydrazones were separated on a X-Terra MS C18 column (2.1 x 250 mm) and analyzed in an Agilent 1100 LC-DAD-MS system (Agilent, Waldbronn) Bruker Esquire 3000 plus (Bruker, Billerica). The mobile phase was water (25%) and acetonitrile (75%). The quantification of the aldehydes, as the respective hydrazones, was done by external calibration curves and the absorbance of compounds was monitored at 365 nm [24].

RESULTS AND DISCUSSION

Quantification and Identification of SRB

The sulfide concentration of the produced water sample was around 20 ppm and the pH was 7.0. The quantification of sulfate-reducing bacteria from the produced water samples by the MPN method showed a low microbial cell density of $2.4x10^3$ cells/ml. This result combined with the low sulfide concentrations measured confirmed the low activity of SRB in the produced water sample. The composed SRB culture APB55 showed the presence of SRB belonging to the family of Desulfovibrionaceae and the genus *Desulfobacter* by using FISH and SRB-385, DSV-698, and SRB-129 probes (Figure 1).

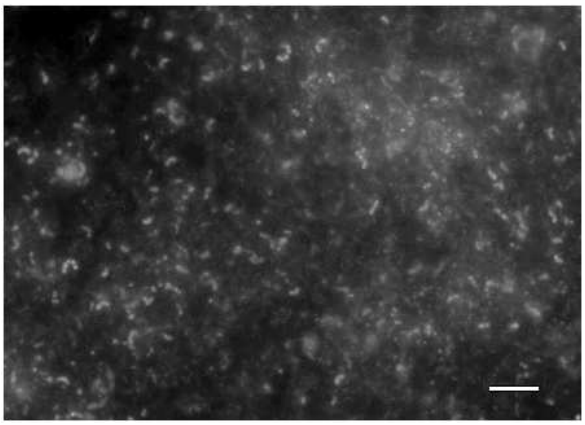

Figure 1: Mixed culture of SRB (APB55) visualized by fluorescence in situ hybridization (FISH) using SRB-385 probe and Cy3 as a fluorochrome; Bar represents 5 µm.

Biogenic Production of Sulfide

The results obtained from APB55 showed that the culture produced sulfide during its growth, when CG was used at concentrations of 1 (data not shown) and 2% (Figure 2A). The sulfide level reached more than 250 ppm for 2% CG, which equated to the complete utilization of the entire sulfate available in the medium. The sulfide production by APB55 with CG originating from soybeans was similar to the control (commercial glycerol) (Figure 2A). However, the initial sulfide production (after 25 hours) was almost two times lower compared to the commercial glycerol. All the other types of CG resulted in a delayed sulfidogenic activity (WOF- and cotton-type CG) of the culture or lowered final concentrations (WOF) of sulfide.

At concentrations of 3% (Figure 2B), 4% (data not shown), and 5% CG (Figure 2C), the biogenic production of sulfide was lowered (castor beans- and soybeans-type CG) or even completely inhibited (WOF- and cotton-type CG). At 3% CG, the sulfide level was reduced from more than 250 ppm (control) to values close to 30 ppm.

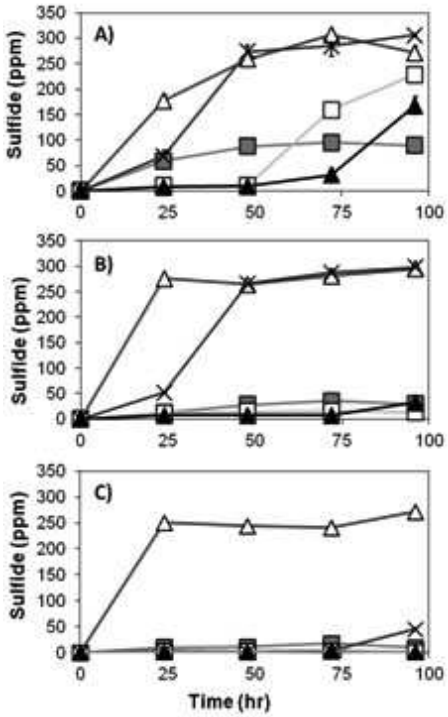

Figure 2: Sulfide production by a mixed culture of sulfate-reducing bacteria (APB55, 10^5 cells/ml inoculum) using crude glycerol from biodiesel production plants using different source materials: ■, castor beans; ▲, cotton; □, waste oils and fats (WOF); X, soybeans; Δ,control (pure glycerol). The concentration of the substrates varied as follows: A) 2%, B) 3%, and C) 5% crude glycerol (w/v). Means ± range (bars), *n* = 2.

Only crude glycerol from soybeans reached the same end concentration of sulfide compared with the control, though having a slower start-up

(Figure 2B). Overall, the inhibition of biogenic sulfide formation was most pronounced at a CG concentration of 5%. Then, sulfide levels were reduced to approximately 10 ppm. Only in cultures with CG from soybeans, the amount of sulfide reached around 50 ppm (Figure 2C).

Moreover, the inoculum density (10^3 and 10^4 cells/ml of APB55 compared to 10^5 cells/ml) had an influence on biogenic sulfate formation (data not shown). The lower the initial cell density was, the higher the inhibitive effect of CG on sulfate reduction became compared to control with commercial glycerol.

Chemical Composition of Crude Glycerol

The concentration of pure glycerol in different crude glycerol samples ranged from 2 to 40 mg/ml (Table 1). Especially cotton- and soybeans-type CG showed very low concentrations of glycerol. These values were below the expected ones reported in literature [2,7]. The lower glycerol content in cotton- and soybeans-type CG samples may be related to the presence of polymers of glycerol formed during the production of biodiesel [25]. These glycerol complexes are probably not, or to a lower degree, reactive in the assay, underestimating the total glycerol concentration. The quantification of the aldehydes using liquid chromatography identified some carbonyl compounds (Table 1). Some of these compounds have earlier been reported to inhibit the growth of SRB such as acrolein and formaldehyde [26-28]. The concentrations of acrolein were highest in CG from soybeans, whereas formaldehyde was most enriched in CG from castor beans (Table 1).

Crude glycerol is a cheap carbon source and could be seen as potential substrate for many biotechnological processes. Next to other applications, crude glycerol has successfully been used for microbial enhanced oil recovery purposes. However, several questions, including which microorganisms are involved and, foremost, what impact such a treatment has on the sulfate-reducing community and consequently on souring, have still remained open.

Table 1: Chemical composition of crude glycerol samples, deriving from biodiesel plants processing different source materials.

Composition (mg/ml)	Crude glycerol			
	Castor Beans	Cotton	Waste oils and fats	Soybeans
Glycerol	20.98	2.57	41.87	2.66
Formaldehyde	0.12	-	0.05	-
Acetaldehyde	1.26	0.11	0.70	0.03
Acrolein	0.10	0.23	0.18	12.60
Propion aldehyde	-	0.63	0.19	0.10
Butiraldehyde	-	0.31	0.16	0.06
Benzaldehyde	-	0.51	-	-
Isovaler aldehyde	-	0.22	0.11	-
Valeraldehyde	-	1.70	0.52	0.21
o-tolualdehyde	-	0.17	0.15	-
m-tolualdehyde	0.20	0.58	-	0.08
p-tolualdehyde	0.04	3.38	0.83	0.29

The main problems of souring are the reduction of the productivity and quality of produced hydrocarbons and increased corrosive processes due to the activity of sulfate reducers [10,11]. In our study, SRB indigenous to oil fields were used in order to mimic the native in situ microbiology and to have a stronger predictive power in this question. Two groups of sulfate reducers were isolated from the produced water and used for further experiments; they belonged to the Desulfovibrionaceae family and the genus *Desulfobacter*. Voordouw et al. [29] observed that all SRB detected from different oil fields using the 16S rRNA gene belong to the Desulfovibrionaceae or Desulfobacteriaceae families. *Desulfovibrio* is a regularly found genus of SRB in produced waters from oil fields and several species have been isolated from this environment [30,31].

The biological conversion of 1 mM glycerol to acetate theoretically reduces 0.75 mM sulfate to sulfide. However, the complete oxidation of glycerol coupled to sulfate reduction reduces 1.75 mM sulfate. Sulfide formation higher than stoichiometrically expected values may also be related to the broad variety of compounds present in CG samples besides glycerol (such as organic acids). These compounds may also contribute as potential electron donors to the reduction of sulfate, and consequently a higher amount of sulfide will be formed compared to the amount stoichiometrically expected from glycerol oxidation only.

In this work, crude glycerol was shown to promote the biological reduction of sulfate to sulfide by a mixed culture of SRB, deriving from an oil field. Several sulfate-reducing bacteria are able to use glycerol as an electron donor. Several *Desulfovibrio* species were reported to grow with glycerol [32-34]. The ability to use glycerol was also demonstrated for *Desulfospira joergensenii*, isolated from marine sediments [35] and members of the genus *Desulfosporosinus* originating from acid sediments [36,37].

Our study shows that increased concentrations of crude glycerol inhibit the biogenic production of sulfide. This could possibly be related to the presence of toxic compounds in CG. The chromatographical analyses of the CG samples identified aldehydes that may be associated with the inhibition of microorganisms, as has been shown for acrolein and formaldehyde [26,27] (Table 1). Acrolein is the main product of dehydration of glycerol during transesterification processes. It is also found in biofuel plants when fats and oils are directly used in catalytic thermolysis reactions [38]. Formaldehyde is the main product formed by the catalytic oxidation of methanol [39], which is usually used in the transesterification process. In this way, both acrolein and formaldehyde can be formed during the biodiesel production. These compounds are also used in biocides to control the growth of gram-positive and gram-negative bacteria, fungi, and yeast [26-28]. Nevertheless, in this study, a direct relationship between the measured concentrations of acrolein and formaldehyde and the inhibition of sulfidogenesis was not observed (comparing Table 1 and Figures 2B/C).

Alkali metals may also have inhibitory effects on microorganisms. The inhibitory concentration of sodium ion (Na^+) is between 3 to 16 g/l [40] and that of potassium ion (K^+) is 2 to 12 g/l [41]. Chaves et al. [42] detected high values of sodium (0.36 to 19 g/l) and potassium (<0.08 to 92 g/l) in the CG samples from vegetable waste oil and commercial soybeans oil. The authors also observed that there was a large variation in the concentrations of Na^+ and K^+ in the CG samples. This indicates that much of the catalyst used is concentrated in the crude glycerol phase. The high concentration of Na^+ and K^+ may be related to the choice of catalyst used in the biodiesel production, which are usually sodium hydroxide (NaOH) or potassium hydroxide (KOH).

We suppose that organic waste compounds from biodiesel production (such as acrolein and formaldehyde) and the high concentration of catalysts (NaOH or KOH) in CG act directly or via an associated way on the metabolism of SRB. This is in line with other studies where crude glycerol (in contrast to pure glycerol) showed to be toxic to *Clostridium pasteurianum* [43-44].

Further studies have to be conducted for a better understanding of the exact inhibitory effect of higher concentrations of CG on the biogenic formation of sulfide.

ACKNOWLEDGEMENTS

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Petróleo Brasileiro S.A., and the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB). The research was part of the Ph.D. program of the Northeast

Brazil Biotechnology Network (RENORBIO).

CONCLUSIONS

A definite prediction about the potential of sulfide formation after CG injection in oil fields is not possible from this study. However the results show that CG is utilized by indigenous SRB and may therefore contribute to an increase in souring of oil reservoirs. A continuous dosing of sufficiently high CG concentrations to inhibit sulfidogenesis seems impracticable and could not be maintained throughout an oil reservoir.

On the other hand, the study suggests CG (at lower concentrations) as a good electron donor for SRB (Figure 2A). CG might, for instance, serve as a cheap and abundantly available source for metal precipitation processes based on biologically formed sulfide such as the processes used for the bioremediation of acid mine drainages and the biorecovery of metals.

REFERENCES

[1] Hansen C. F., Hernandez A., Mullan B. P., Moore K., et al., "A Chemical Analysis of Samples of Crude Glycerol from the Production of Biodiesel in Australia, and the Effects of Feeding Crude Glycerol to Growing-finishing Pigs on Performance, Plasma Metabolites and Meat Quality at Slaughter," *Anim. Prod. Sci.*, **2009**, *49*, 154-161.

[2] Hazimah A. H., Ooi T. L., and Salmiah A., "Recovery of Glycerol and Diglycerol from Glycerol Pitch," *J. Oil Palm Res.*, **2003**, *15*, 1-5.

[3] Sandun F., Sushil A., Kiran K., and Ranjitha B., "Glycerol Based Automotive Fuels from Future Biorefineries," *Fuel*, **2007**, *86*, 2806-2809.

[4] Yazdani S. S. and Gonzalez R., "Anaerobic Fermentation of Glycerol: a Path to Economic Viability for the Biofuels Industry," *Curr. Opin. Biotechnol.*, **2007**, *18*, 213-219.

[5] Johnson D. T. and Taconi K. A., "The Glycerin Glut: Options for the Value-added Conversion of Crude Glycerol Resulting from Biodiesel Production," *Environ. Progress*, **2007**, *2*, 338-348.

[6] Silva G. P., Mack M., and Contiero J., "Glycerol: A Promising and Abundant Carbon Source for Industrial Microbiology," *Biotechnol. Adv.*, **2009**, *27*, 30-39.

[7] Quintella C. M., Borges S. M. S., Almeida P. M. M., Musse A. P. S., et al., "Secondary Recovery of Crude Oil with Glycerin, Co-product of the Biodiesel Production," *Anais Rio Oil & Gas Expo. and Conference*, Rio de Janeiro, **2006**.

[8] Muyzer G. and Stams A. J. M., "Ecology and Biotechnology of Sulphate-reducing Bacteria," *Nature Rev. Microbiol.*, **2008**, *6*, 441-454.

[9] Postgate J. R., *The Sulphate-reducing Bacteria*, Cambridge: Cambridge University Press, **1984**.

[10] Hubert C., Nemati M., Jenneman G., and Voordouw G., "Corrosion Risk Associated with Microbial Souring Control Using Nitrate or Nitrite," *Appl. Microbiol. Biotechnol.*, **2005**, *68*, 272-282.

[11] Coetser S. E. and Cloete T. E., "Biofouling and Biocorrosion in Industrial Water Systems," *Crit. Rev. Microbiol.*, **2005**, *31*, 213-232.

[12] Estublier A., Dino R., Schinelli M. C., Barroux C., et al., "CO_2 Injection in Buracica-long-term Performance Assessment," *Energy Procedia.*, **2011**, *4*, 4028-4035.

[13] American Society of Testing and Material, *Standard Test Method for Sulphate-reducing Bacteria in Water and Water-formed Deposits*, West Conshohocken: American Society of Testing and Material Annual Book of Standards (ASTM, D4412), **2002**.

[14] *American Public Health Association, Standard Methods for the Examination of Water and Wastewater* (13th ed.), Washington: American Public Health Association, **1973**.

[15] Clesceri S., Greenberg A. E., and Eaton A. D., *Standard Methods for the Examination of Water and Wastewater* (20th ed.),

Washington: American Public Health Association, American Water Works Association, Water Environmental Federation, **1998**.

[16] Daims H., Stöcker K., and Wagner M., Fluorescence In Situ Hybridization for the Detection of Prokaryotes, in *Advanced Methods in Molecular Microbial Ecology*, Osborn A. M., Smith C. J., ed. Abingdon: BIOS Scientific Publishers, **2005**, 213-239.

[17] Amann R. I., Devereux R., Key R., and Stahl D. A., "Molecular and Microscopic Identification of Sulfate-reducing Bacteria in Multispecies Biofilms," *Appl. Environ. Microbiol.*, **1992**, *58*, 614-623.

[18] Manz W., Eisenbrecher M., Neu T. R., and Szewyk U., "Abundance and Spatial Organization of Gram-negative Sulfate Reducing Bacteria in Activated Sludge Investigated by In Situ Probing with Specific 16S rRNA Targeted Oligonucleotides," *FEMS Microbiol. Ecol.*, **1998**, *25*, 43-61.

[19] Devereux R., Kane M. D., Winfrey J., and Stahl D. A., "Genus and Group-specific Hybridization Probes for Determinative and Environmental Studies of Sulfate Reducing Bacteria," *Syst. Appl. Microbiol.*, **1992**, *15*, 601-609.

[20] Santos A. F. J., Batista L. L. F., Lima J. B. T., Almeida R. C. C., et al., "Evaluation of the Fluorescence in Situ Hybridization Technique for Detection of Eubacteria and Sulfate-reducing Bacteria from Samples of Water in Oil Fields," *Chem. Eng. Trans.*, **2010**, *20*, 139-144.

[21] Gherna R. L., "Preservation," in *Manual of Methods for General Microbiology, Gerhard P.*, ed. Wshington: American Society for Microbiology, **1981**, 208-217.

[22] Francisco D. E., Mah R. A., and Rabin A. C., "Acridine Orange Epifluorescence Technique for Counting Bacteria in Natural Waters," *Trans. Am. Microsc. Soc.*, **1973**, *92*, 416-421.

[23] Trüper H. G. and Schlegel H. G., "Sulfur Metabolism in Thiorhodeaceae. I. Quantitative Measurements on Growing Cells of Chromatium Okenii," *Antonie van Leeuwenhoek*, **1964**, *30*, 225-238.

[24] Bastos L. C. S. and Pereira P. A. P., "Influence of Heating Time and Metal Ions on the Amount of Free Fatty Acids and Formation Rates of Selected Carbonyl Compounds During the Thermal Oxidation of Canola Oil," *J. Agric. Food Chem.*, **2010**, *58*, 12777-12783.

[25] "Journal of Plastic," Internet: www.jorplast.com.br, Nov. 9, 2010 [Nov. 9, 2010].

[26] Feron V. J., Til H. P., de Vrijes F., Wouterson R. A., et al., "Aldehydes: Occurrence, Carcinogenicity Potential, Mechanism of Action and Risk Assessment," *Mutat. Res.*, **1991**, *259*, 363-385.

[27] Ma T. H. and Harris M. M., "Review of the Genotoxicity of Formaldehyde," *Mutat. Res.*, **1988**, *196*, 37-59.

[28] Fink T.R. and Worner P., "Pollution Prevention Opportunities in Oil and Gas Production, Drilling, and Exploration," Internet: http://infohouse.p2ric.org/ref/03/02975.pdf, **1993** [Dec. 23, 2014].

[29] Voordouw G., Armstrong S. M., Reimer M. F., Fouts B., et al., "Characterization of 16S rRNA Genes from Oil Field Microbial Communities Indicates the Presence of a Variety of Sulfate-reducing, Fermentative, and Sulfide-oxidizing Bacteria," *Appl. Environ. Microbiol.*, **1996**, *62*, 1623-1629.

[30] Magot M., Basso O., Tardy-Jacquenod C., and Caumette P., "Desulfovibrio Bastinni sp. nov. and Desulfovibrio Gracilis sp. nov., Moderatety Halophilic, Sulfate-reducing Bacteria Isolated from Deep Subsurface Oilfield Water," *Int. J. Syst. Evol. Microbiol.*, **2004**, *54*, 1693-1697.

[31] Miranda-Tello E., Fardeau M. L., Fernandéz L., Ramiréz F., et al., "Desulfovibrio Capillatus sp. nov., a Novel Sulfate-reducing Bacterium Isolated from an Oil Field Separator located in the Gulf of Mexico," *Anaerobe*, **2003**, *8*, 97-103.

[32] Stams A. J. M., Hansen T. A., and Skyring G. W., "Utilization of Amino Acids as Energy Substrates by Two Marine Desulfovibrio Strains," *FEMS Microbiol. Ecol.*, **1985**, *31*, 11-15.

[33] Kremer D. R. and Hansen T. A., "Glycerol and Dihydroxyacetone Dissimilation in Desulfovibrio Strains," *Arch. Microbiol.*, **1987**, *147*, 249-256.

[34] Esnault G., Caumette P., and Garcia J. L., "Characterization of Desulfovibrio Giganteus sp. nov., a Sulfate-reducing Bacterium Isolated from a Brackish Coastal Lagoon," *Syst. Appl. Microbiol.*, **1988**, *10*, 147-151.

[35] Finster K., Coates J. D., Liesack W., and Pfennig N., "Desulfuromonas Thiophila sp. nov., a New Obligately Sulfur-reducing Bacterium from Anoxic Freshwater Sediment," *Int. J. Syst. Bacteriol.*, **1997**, *47*, 754-758.

[36] Alazard D., Joseph M., Battaglia-Brunet F., Cayol J. L., et al., "Desulfosporosinus Acidiphilus sp. nov.: a Moderately Acidophilic Sulfate-reducing Bacterium Isolated from Acid Mining Drainage Sediments," *Extremophiles*, **2010**, *14*, 305-312.

[37] Sánchez-Andrea I., Stams A. J., Amils R., and Sanz J. L., "Enrichment and Isolation of Acidophilic Sulfate-reducing Bacteria from Tinto River Sediments," *Environ. Microbiol. Reports.*, **2013**, *5*, 672-678.

[38] Parshall G. W. and Ittel S. D., "Homogeneous Catalysis: the Application and Chemistry of Catalysis by Soluble Transition Metal Catalysts," New York: John Willey and Sons, **1992**.

[39] Allison J. N., Goddard III W. A., "Oxidative Dehydrogenation of Methanol to Formaldehyde," *J. Cat.*, **1985**, *92*, 127-135.

[40] Feijoo G., Soto M., Mendez R. E., and Lema J. R., "Sodium Inhibition in the Anaerobic Digestion Process: Antagonism and Adaptation Phenomena," *Enz. Microbial. Technol.*, **1995**, *17*, 180-188.

[41] Bashir B. H. and Matin A., "Combined Effect of Calcium and Sodium on Potassium Toxicity in Anaerobic Treatment Processes," *Electron. J. Environ. Agric. Food Chem.*, **2004**, *4*, 670-676.

[42] Chaves E. S., Ramos J. C., Fontana K. B., Modolon S., et al., "Simple and Fast Method for the Determination of Na and K in Raw Glycerin from Biodiesel Production by Flame Atomic Emission Spectrometry," *Br. J. Anal. Chem.*, **2010**, *1*, 24-59.

[43] Jensen T., Kvist T., Mikkelsen M. J., Christensen P. V., et al., "Fermentation of Crude Glycerol from Biodiesel Production by Clostridium Pasteurianum," *J. Ind. Microbiol. Biotechnol.*, **2012**, *39*, 709-717.

[44] Dabrock B., Bahl H., and Gottschalk G., "Parameters Affecting Solvent Production by Clostridium Pasteurianum," *Appl. Environ. Microbiol.*, **1992**, *58*, 1233-1239.