



## Indirect determination of nitrogen oxides produced in a nitrogen-generating system by RGB image analysis using the Griess assay

Marcos Vinicius Miranda Mesquita<sup>1</sup>, Alline V. B. de Oliveira<sup>1</sup>, Felipe J. S. Bispo<sup>1</sup>, Ronald Wbeimar Pacheco Ortiz<sup>1</sup>, Vinicius Ottonio O. Gonçalves<sup>1</sup>, Vinicius Kartnaller<sup>1</sup>, and João Cajaiba<sup>1\*</sup>

<sup>1</sup>Center of Development of Processes and Chemical Analysis in Real Time, Institute of Chemistry, Federal University of Rio de Janeiro. \*cajaiba@iq.ufrj.br

### Abstract

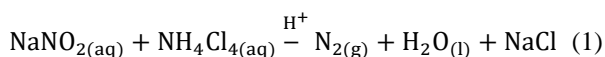
A nitrogen-generating system (NGS) is an acid-catalyzed exothermic reaction used to fluidize low-melting-point deposits occurring in subsea oil production. The acid catalyst can also decompose the nitrite to produce nitrogen oxides (NO<sub>x</sub>), which are toxic and pollutant gases whose emissions must be controlled. This work presents the indirect determination of NO<sub>x</sub> in the form of nitrite using the Griess assay. Nitrite solutions were mixed with the Griess reagents (sulfalinic acid and N,N-dimethylaniline) to produce a reddish/purplish azo dye. The color temporal stability was evaluated and a pH of 5 was suitable for preserving the color. The colorful solution was then analyzed by spectrophotometry (470 nm) and RGB image analysis to obtain analytical curves to quantify indirectly NO<sub>x</sub>, which for the RGB image analysis was the variation of G. This method was applied to determine the concentration of the NO<sub>x</sub> produced in a NGS, which was carried out by mixing aqueous solutions of sodium nitrite and ammonium chloride using either acetic acid or CO<sub>2</sub> (carbonic acid precursor) as acid catalyst. With a confidence interval of 95%, the concentration of NO<sub>x</sub> determined by both techniques was the same. Thus, the image analysis becomes a low-cost alternative to quantify NO<sub>x</sub> using the Griess assay.

### Keywords

Low-melting-point deposit; Pollutant gas; Spectrophotometry

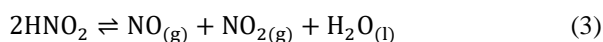
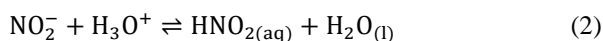
### Introduction

A nitrogen-generating system (NGS) is a method used for the remediation of low-melting-point deposits such as paraffin and gas hydrates in offshore oil production. This is an exothermic reaction between aqueous solutions of nitrogenous salts (e.g. sodium nitrite and ammonium chloride) catalyzed by acids as shown in Eq. (1) [1].



$$\text{With } \Delta H = 322.92 \frac{\text{kJ}}{\text{mol N}_2}$$

Meanwhile, the acid catalyst can also decompose the nitrite into nitrogen oxides (NO<sub>x</sub>) according to Eq. (2) and Eq. (3).



NO<sub>x</sub> is an important group of air pollutants mainly of anthropogenic origin. They are responsible for numerous negative impacts on human health and the environment: low air quality, climate change, ecological imbalance, and so on. Therefore, the

development of analytical strategies to quantify NO<sub>x</sub> is important to control their emissions [2].

Direct determination of NO<sub>x</sub> is usually performed by chemiluminescence [3], whereas the indirect one (with previous conversion to nitrite and/or nitrate) can be performed by various techniques: ion chromatography, potassium permanganate titration, and spectrophotometry [2]–[5].

Spectrophotometry is the most used technique and is based on the formation of an azo dye using the Griess assay. The Griess assay is the reaction between nitrite and sulfanilamide/sulfalinic acid under acidic conditions to form a diazonic cation, which then reacts with an aromatic amine (e.g. N,N-dimethylaniline) to produce a reddish/purplish water-soluble azo dye [6].

The formation of this colorful substance also allows the application of image analysis, which is a faster and less expensive technique than spectrophotometry, to indirectly quantify the NO<sub>x</sub>. Image analysis uses the CCD or COMS sensor of a camera to acquire colorful images using an RGB (red, green, and blue) matrix filter. The RGB values can be then related to the concentration of NO<sub>x</sub> [7]. Thus, this work aims to use the Griess assay for the indirect determination of NO<sub>x</sub> produced in a NGS catalyzed either by acetic acid or CO<sub>2</sub>

(carbonic acid precursor), comparing the spectrophotometry and image analysis techniques.

## Methodology

### Griess assay analytical curves

#### Color temporal stability at variable pH:

To evaluate the color stability of the azo dye at variable pH, 1 mL of a sulfalanic acid solution 1% in acetic acid 30% (Reagent 1) was mixed with 1 mL of a N,N-dimethylaniline solution 0.3% in acetic acid 30% (Reagent 2). After 5 min this mixture turned reddish and a pH of 1 was determined using a pH test strip. The same procedure was carried out but adding 1 mL of an ammonium hydroxide solution 6 mol/L and after 5 min the color was orangish and the pH was 5. The absorbance of both mixtures was then measured for 60 min using a spectrophotometer Ocean Optics USB2000+UV-VIS-EIS, at 480 nm for the product obtained at pH 1 and at 470 nm for the one obtained at pH 5.

#### Analytical curves to quantify nitrite:

Five solutions of sodium nitrite were prepared from a stock solution of  $8.0 \times 10^{-5}$  mol/L in test tubes. The concentration of these solutions varied from  $1.6 \times 10^{-5}$  to  $8.0 \times 10^{-5}$  mol/L and their final volume was 1 mL. Each solution was then mixed with 1 ml of both Reagent 1 and Reagent 2 at room temperature and after 5 min was added 1 mL of ammonium hydroxide 6 mol/L. For the blank test, the nitrite solution was replaced by distilled water. The absorbance of all the solutions was measured at 470 nm. The color of these solutions was also measured by image analysis but adding other five solutions within the same concentration range to enhance the correlation. Figure 1 shows a schematic representation of the image analysis apparatus, which was a metallic box internally lined with white cardstock paper, equipped with a webcam (Microsoft LifeCam Cinema 720p HC) and a LED (light-emitting diode) with luminous flux 280 lm. The webcam and the lamp were positioned at a 45° angle regarding the sample. The sample tube was placed inside the box through an access in the top. The RGB values were obtained using the software RGBview®.

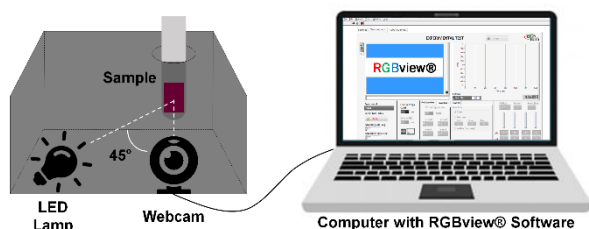
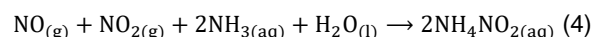


Figure 1. Schematic representation of the image analysis apparatus

### Indirect determination of NO<sub>x</sub> produced in a Nitrogen-generating system

The nitrogen generating system (NGS) was carried out in a jacketed stainless-steel batch reactor (Midiclave, Büchiglasuster, Switzerland) of 500 mL

equipped with a temperature sensor (PT100), pressure sensor, and mechanical stirrer. The temperature was controlled by a thermostatic bath (Unistat Tango, Huber). 100.00 mL of sodium nitrite solution 8 mol/L and 177.70 mL of ammonium chloride solution 4 mol/L were added to the reactor set at 15.5°C and 350 rpm. Then, the acid catalyst was added to the system, which was either 22.30 mL of acetic acid 1.75 mol/L or 40 bar of CO<sub>2</sub>. After 40 min of reaction, the gaseous phase of the reactor was bubbled into 200 mL of sodium hydroxide solution 6 mol/L to absorb the NO<sub>x</sub> produced in the form of nitrite according to Eq. (4) [8].



The gas-phase collection ended when the reactor pressure was atmospheric. The pH of the absorbent solution was adjusted to 7 with sulfuric acid and then it was diluted to 250 mL with Milli-Q® water. 1 ml of this solution was used to perform the Griess assay by both spectrophotometry and image analysis.

## Results and Discussion

The results presented here are the concentration of nitrite, which allows the indirect determination of NO<sub>x</sub> according to Eq. (4). Figure 2 shows the stability of the color at pH 1 and 5. pH 1 is the resulting pH of the Griess reagents mixture since an acid medium is required for this reaction. However, the intensity of the reddish color of the azo dye increased over time, which affected the spectrometric result shown in Fig. (2). Therefore, the Griess assay was performed at a higher pH (5), where it was obtained a more time stable orangish color as observed in Fig. (1). After 60 min, the absorbance of the product at pH 1 increased 11-fold, whereas increased only 1-fold for pH 5. Therefore, pH 5 was always used in the following stages of this work.

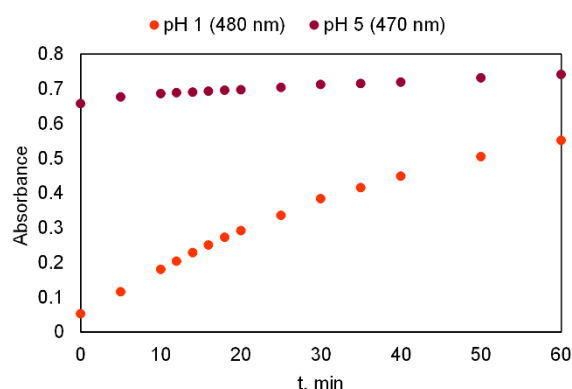


Figure 2. Evaluation of the temporal stability of the Griess assay azo dye color

Figure 3 displays the analytical curve to quantify nitrite by spectrophotometry at 470 nm. This curve presents the expected linear relationship between concentration and absorbance ( $R^2 = 0.9895$ ),

which indicates that the nitrite concentration range used here is suitable without requiring further dilution. Moreover, this curve was performed at pH 5, which ensures the reliability of the results and a suitable comparison with the image analysis.

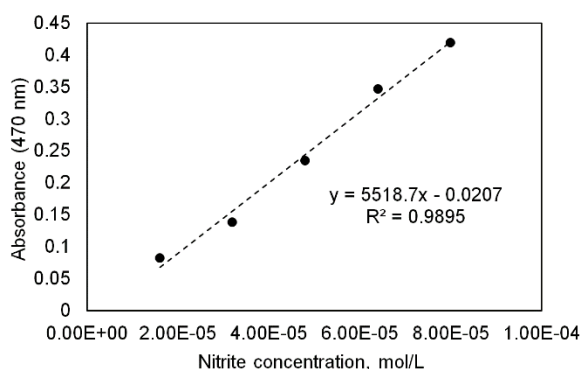


Figure 3. Analytical curve to quantify nitrite by spectrophotometry using the Griess assay

Figure 4 shows the nitrite solutions images obtained by the image analysis apparatus and the corresponding RGB values. The RGB color space is measured between 0 and 255, where 255 is the white color and 0 is the black one [9]. Thus, the blank test, which is a colorless solution, presents the maximum RGB values that correspond to the white color of the internal walls of the box. The RGB values then decrease with the increasing orangish color intensity, and therefore with the increasing nitrite concentration.

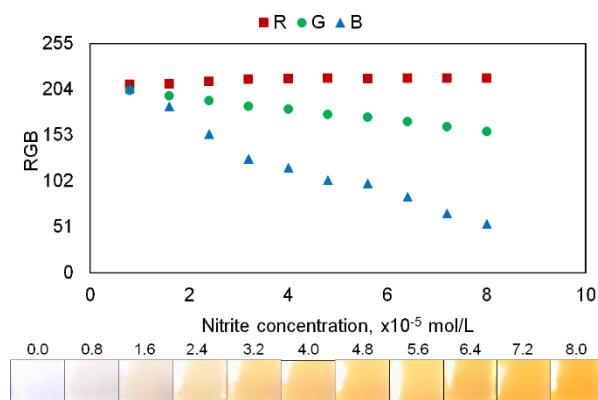


Figure 4. RGB image analysis of the nitrite solutions

Thus, to obtain a RGB image analysis response similar to the absorbance that increases with the increasing nitrite concentration, it was decided to use the variation the RGB values ( $\Delta$ RGB). Thus, it was obtained the analytical curves presented in Fig. (5), where the variation of the green component ( $\Delta$ G) shows the best linear relationship ( $R^2 = 0.9964$ ) with the nitrite concentration. Therefore,  $\Delta$ G was selected to determine the  $\text{NO}_x$  concentration by image analysis.

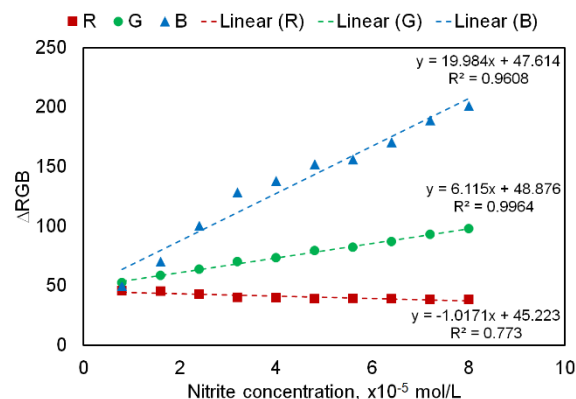


Figure 5. Analytical curves to quantify nitrite by image analysis using the Griess assay

The production of  $\text{NO}_x$  in the NGS under the experimental conditions used in this work was confirmed since the nitrite formed in the absorbent ammonium solution was detected using the Griess assay. As explained before, the  $\text{NO}_x$  can be indirectly quantified from the nitrite concentration (Eq. 4), and Table 1 shows the results.

Table 1. Concentration of the  $\text{NO}_x$  produced in a nitrogen-generating system

Catalyst	$\text{NO}_x$ concentration, mol/L	
	Spectrophotometry	Image Analysis
Acetic acid	$(4,9 \pm 1,8) \times 10^{-4}$	$(5,6 \pm 1,9) \times 10^{-4}$
$\text{CO}_2$	$(1,6 \pm 0,4) \times 10^{-4}$	$(1,7 \pm 0,5) \times 10^{-4}$

Considering a confidence interval of 95 %, it is possible to affirm that the results obtained by both techniques are equal. Moreover, the production of  $\text{NO}_x$  was lower in the NGS catalyzed by  $\text{CO}_2$ , which is a very interesting result that can be further investigated to turn this process safer and more environmentally friendly than the one catalyzed by conventional acid catalysts [10].

## Conclusions

This work presents the indirect determination of nitrogen oxides ( $\text{NO}_x$ ) using the Griess assay by both spectrophotometry (at 470 nm) and RGB image analysis (variation of the green component). This method was also applied for the determination of the  $\text{NO}_x$  produced in a nitrogen-generating system (NGS) catalyzed by either acetic acid or  $\text{CO}_2$  (carbonic acid precursor). The analytical techniques presented statistically equal results, with a confidence interval of 95%. Therefore, as the image analysis is faster and less expensive than spectrophotometry, becomes an interesting alternative for the indirect determination of the  $\text{NO}_x$  using the Griess assay. The application of this method for quantifying the  $\text{NO}_x$  produced in a NGS demonstrates that is suitable for monitoring and controlling the emissions of these toxic and pollutant gases.

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## Responsibility Notice

The authors are the only responsible for the paper content.

## References

- [1] V. Kartnaller, D. C. O. Mariano, and J. Cajaiba, "Application of In-Line Mid-Infrared (MIR) Spectroscopy Coupled with Calorimetry for the Determination of the Molar Enthalpy of Reaction between Ammonium Chloride and Sodium Nitrite," *Appl. Spectrosc.*, 70 (3), 531–538, 2016.
- [2] F. J. S. Bispo, V. Kartnaller, and J. Cajaiba, "Controlling Nitrogen Oxide (NO<sub>x</sub>) Emissions from Exothermic Nitrogen Generation Systems for Application in Subsea Environments," *ACS Omega*, 4 (26), 21985–21992, 2019.
- [3] L. Bellavia, D. B. Kim-Shapiro, and S. B. King, "Detecting and monitoring NO, SNO and nitrite in vivo," *Futur. Sci. OA*, 1 (1), 2015.
- [4] B. E. Saltzman, "Colorimetric Microdetermination of Nitrogen Dioxide in the Atmosphere," *Anal. Chem.*, 26 (12), 1949–1955, 1954.
- [5] M. Nonomura, T. Hobo, E. Kobayashi, T. Murayama, and M. Satoda, "Ion chromatographic determination of nitrogen monoxide and nitrogen dioxide after collection in absorption bottles," *J. Chromatogr. A*, 739 (1–2), 301–306, 1996.
- [6] P. Griess, "Bemerkungen zu der Abhandlung der HH. Weselsky und Benedikt „Ueber einige Azoverbindungen“,“ *Berichte der Dtsch. Chem. Gesellschaft*, 12 (1), 426–428, 1879.
- [7] F. Venâncio, F. F. do Rosário, and J. Cajaiba, "A low-cost system based on image analysis for monitoring the crystal growth process," *Sensors*, 17 (6), 1–6, 2017.
- [8] W. Braida and S. K. Ong, "Decomposition of nitrite under various pH and aeration conditions," *Water. Air. Soil Pollut.*, 118 (1–2), 13–26, 2000.
- [9] A. V. B. De Oliveira, R. W. P. Ortiz, V. Kartnaller, F. Venancio, V. O. O. Goncalves, and J. Cajaiba, "Real-Time Measurement of pH in Atmospheric and Pressurized Systems Using a Low-Cost Image Analysis Method," *IEEE Sens. J.*, 19 (23), 10991–10998, 2019.
- [10] M. V. M. Mesquita *et al.*, "Evaluation of CO<sub>2</sub> as an Acid Catalyst Precursor for Promoting a Nitrogen-Generating System," *SPE J.*, 1–8, 2022.