Comparative analysis of bio fouling microorganisms after treatment with glidarc

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Abstract. The biofouling of the surfaces immersed in water is one of the most important problems which must be solved in the naval field. This phenomenon is amplified during the harbor operations, when the ships are stationary and there is a high density of microorganisms in the neritic area, developing the biofouling. For this reason, in order to prevent or delay the deposition of the first layers of biofouling, different methods have been used [1]. This paper presents the comparative analysis of microorganisms’ behavior obtained by using GlidArc technology for the treatment of the naval metallic surfaces [2, 3]. The main parameters identified for data processing were: the number of microorganisms shared from the point of view of sensibility to the used technology found on the metallic surfaces, the type of the naval paint and the treatment methods. For analysis it was used the epifluorescence microscopy method with. The comparative analysis follows the data processing which has the same input characteristics. Finally it was observed the microorganism’s lifetime after the surface treatment.

1. Introduction
Any naval treatment should not affect the sea biodiversity. The biofouling is a well known problem, involving environmental impact. Its consequences are hardly important on naval corrosion topic. Marine biofouling is typically described by following four stages of ecosystem development: the first two stages, also named “microfouling”, are referring to the forming condition of the biofilm, related first to the processes of van der Waals interaction covering the submerged surface with a conditioning film of organic polymers, and then of bacterial adhesion (in the next 24 hours); the third concerns the appearance by the end of the first week of secondary colonizers like spores, micro algae, protozoans, etc.; and the last stage is manifested after around 2-3 weeks by the appearance of tertiary colonizers, referring to “macrofouling”.

In this paper is presented a laboratory study on the naval steel samples, using GlidArc as biofouling treatment method. The naval steel was covered with different paints before the experiment. It was studied the microorganism’s evolution in time after the GlidArc treatment. The correlation and ANOVA methods were applied to the results in order to identify the efficient methods for treating and cleaning the naval surfaces.

Gliding Arc discharges (GlidArc, see figure 1) provide the most effective treatments among all types of electrical discharges. They are produced between at least two metallic electrodes with divergent shape structure. In our experiments the electrodes were supplied from a voltage power supply (10kV, 50Hz) providing the maximum electrical current value of 50mA. The minimum...
distance between the electrodes was 2 mm, the maximum distance was 30 mm and the height of the electrodes was 80 mm. The maximum gas flow amount was 50 Nm$^3$/min.

![Figure 1. Photo of GlidArc discharges.](image)

2. Methodology
The direct and indirect treatments with plasma produced by GlidArc are applied on the target surface. The direct treatment consists in applying the plasma electrical discharge direct on the metallic surface. The indirect process consists on activating the water with GlidArc discharge then immersing the metallic samples into this solution.

2.1. Experimental
The experiment consists in two parts depending on the types of samples. The process monitoring is achieved using an epifluorescence trinocular microscope, 400-FL type with blue filter of (450-480) nm. For cells quantification and measurement are used the CellC and Image J software. For each experiment, after treatment, the samples are immersed in sea water.

a. The initial experiment was achieved on untreated samples of naval steel, [2]. It is applied the plasma at the distance of 2.5 cm distance lasting of 5 minutes and at the distance of 1.2 cm during 3.5 minutes. One untreated sample was taken as control to compare the microorganisms’ evolution. The microorganisms are observed using 40 objective x10 eyepiece magnitude microscope. All the data are recorded at the same moments of time: 30 min, 5h, 10h, 24h. With Image J software are achieved 100 measurements/time. The figure 2 depicts the microorganism evolution in time where the green cells represent the living fraction and the red cells represent the dead fraction.

b. The second experiment used three pretreated samples, [3], using different paints during 5 minutes. For the cells quantification we used the CellC software and 20 microscopic fields/samples were measured.

The activated water consists in 50 mL of distilled water exposed to the non-thermal plasma produced by GlidArc. The time of plasma application for the both methods was 8 minutes.

The adherence period consist in immersion samples in sea water during 6h respectively 24h for biofilm coating. The evolution of microorganisms was studied by harvesting samples at 10, 120, 480 and 1440 minutes after the treatment and processed with epifluorescence microscope. The data results indicate the general microorganisms’ evolution and in particular the families as: green algae, cianobacteria and heterotrophic bacteria.
2.2. Data analysis
As methods to identify the microorganism’s behaviors function of GlidArc treatment and the type of surface, the correlation and variance analysis (ANOVA) were used. The correlation describes the behaviors of dead cells in time for the two groups of data and analysis the variance test of significant differences between class means. The ANOVA test is based on comparing two independent estimates of the population variance. ANOVA procedure requires the following assumptions: the observations are independent; the observations in each group come from normal distribution; the population variances in each group are the same [6]. The groups were represented by the samples (red-1, blue-2, and black-3) and the factor by the type of treatment: direct GlidArc (1) or plasma activated water (2). The One-Way ANOVA methods are applied in this case because there is one factor to test the difference between the groups of treatment.

3. Data processing
After treating the naval steel samples, see section 2.1.a, the evolution of dead and alive cells occurred (figure 3). The dead cells on the treated samples have a linear evolution between 5 and 600 min then the evolution of microorganisms are constant up to 24h. The GlidArc is efficient just for a short time period.

The dead cells evolutions reported to the total number of cells in case of 5 min GlidArc exposure are: 76%, 87.3%, 97.5% and 97.4%. It was observed that after 24h the number of dead cells is constant. To observe the GlidArc effect in the both cases it is achieved a correlation between the dead cells. The Pearson Coefficient is 0.989, very closed to 1 so there is a correlation between the responses. In both cases the most of the microorganisms are destroyed until 24h.

The data analysis was continued for the experiments described in section 2.1.b. where the samples were pretreated with different solutions. It was found how the treatment of the samples influences the microorganisms’ evolution using the variance analysis intragroup and intergroup. It was identified the microorganisms’ evolution, of the first layer of biofouling, for the samples immersed in sea water 6h before the test. Because the microorganisms have not the same evolution, they were split in permeabilized heterotrophic bacteria, cyanobacteria and green algae noted with 5, 6 and 7. It was kept the control on each sample to compare the results noted with 11. The samples are noted 1, 2 and 3 for red, blue, black surface (different types of paints), see figure 4.
The evolution of the microorganism in case of the control sample is constant while in the case of treated samples the number of dead cells is increasing. The number of dead cells for the monolayer biofouling decreases after 8h showing that the effect of GlidArc discharge is finished. The microorganisms were split in three groups in order to observe the treatment effect, see table 1. It was used the notation of the samples type/x, where x can be 5, 6, 7 for the microorganisms group.

**Table 1. Microorganisms group correlation function of samples type (direct discharge exposure).**

|                     | 2/x-3/x | 1/x-2/x | 1/x-3/x |
|---------------------|---------|---------|---------|
| Pearson Correlation for 5 | 0.97911 | 0.99408 | 0.9954  |
| Pearson Correlation for 6 | 0.63487 | 0.98332 | 0.76481 |
| Pearson Correlation for 7 | 0.47032 | -0.98143 | -0.29231 |

The heterotrophic cells evolution depends on the paint. For red samples the data are negative correlated with the data of blue and black samples. The heterotrophic cells are influenced by both the paint type and the GlidArc treatment. The green algae and heterobacteria are independent by the samples treatment, being autotrophic. The cleaning process of biofouling is efficient for the monolayer so the data analysis was continued for the first two layers (see section 2.1b). They were kept the same notations and for the independent factor it was marked the direct GlidArc discharge process with 1 and the tests based on activated water with 2.

Using the same principle as for the analysis described above, it was analyzed the microorganisms time evolution for the two treatment types particularized for three types, see table 2.

**Table 2. The correlation of the microorganism’s evolution in the case of two treatments (1 and 2).**

| Samples type/treatments | Pearson corr. 1/1 | Pearson corr. 1/2 | Pearson corr. 2/1 | Pearson corr. 2/2 | Pearson corr. 3/1 | Pearson corr. 3/2 |
|-------------------------|-------------------|-------------------|-------------------|-------------------|------------------|------------------|
| Pearson corr. 1/1       | 1                 | 0.35855           | 0.75042           | 0.50536           | 0.80438          | 0.71148          |
| Pearson corr. 1/2       | 0.35855           | 1                 | 0.87698           | 0.98071           | 0.0031           | 0.8327           |
| Pearson corr. 2/1       | 0.75042           | 0.87698           | 1                 | 0.92508           | 0.46219          | 0.9745           |
| Pearson corr. 2/2       | 0.50536           | 0.98071           | 0.92508           | 1                 | 0.09111          | 0.85442          |
The table 2 indicates the correlation results applied between all the data for each sample submitted to the direct treatment and to the plasma activated water. The microorganisms treated with first methods do not have the same evolution in time as the second. The second method is stronger as the first. There is the intergroup correlation but the results are uncorrelated into the intragroup. The data analyses on group of microorganisms (5, 6, 7) for direct treatment and plasma activated water treatment are achieved with ANOVA method [4].

The factor of analysis is the treatment type: direct and indirect. This is applied for the same group of samples (1, 2, 3). The null hypothesis for the analysis is: the treatment for the entire samples group has the same efficiency. The alternative hypothesis is: the effects of treatments are different for each group. The results show that all of them are significantly drawn for normally distributed population p≥0, 05 (the significance level).

a. Using ANOVA One-Way methods for green algae it was obtained factor value F=2.57861, Prob>F is 0.0629. The factor value F in Distribution Tables for α=0.05 and freedom degree df= (5, 18) is Ftable = 2.7729. In this case the null hypothesis are accepted because F is less than Ftable and p>0.05. There are minor differences between the samples treatment, the Sig Flag = 0 confirming the assumption.

b. In case of cyanobacteria (noted by 6) the ANOVA test applied for the treatment groups in case of each samples shows that F value is 12.70451; Prob>F is 2.20728E-5. Using the F Distribution Tables for the α=0.05 (significance level) and for df = (5, 18) the Ftable = 2.7729. So there is a significant difference between groups because prob computed <0.05 and F computed is bigger than Ftable, showing that the null hypothesis is rejected. There is a difference between the data of the same group and between the different groups. In the case of cyanobacteria the evolution depends on the treatment and the samples type. It was observed that the mean difference between samples intergroup is negative for (3/2, 1/2) and (2/2, 1/2), indicating that the red samples have inactivated the cyanobacteria less than the other samples in the both cases of treatment.

c. The permeabilized heterotrophic bacteria results obtained by ANOVA method are F=55.67227 and rob=2.55018E-10, for the same table parameter Ftable for α=0.05 significance degree, df= (5, 18). The F factor is bigger than Ftable and the probability factor is inferior to α. The null hypothesis are rejected and there is a significant difference between heterotrophic bacteria evolution function of the type of treatment and samples pretreatment (figure 5).

The heterotrophic cells inactivation is very sensitive for both types of treatment. There is a big difference behavior between the cells intergroup and intragroup. Consequently these types of cells have unusual evolution compared with the other described above. Difference in response to GlidArc treatment between heterotrophic and autotrophic (cyanobacteria) cells can be attributed to structural differences of the cell wall.

![Figure 5](image.png)

**Figure 5.** The means of groups, the significant and no significant difference for the permeabilized heterotrophic bacteria evolution.
Prokaryotic cells contain the peptidoglycan layer and this polymer was found in cyanobacteria being considerably thicker than that of most gram-negative bacteria. The unique structure of prokaryotic cell walls makes bacteria vulnerable to decontamination methods.

4. Conclusions
The microorganisms’ time evolution has the same results for all the experimental methods in the case of the direct action. For the untreated samples the GlidArc acts in the first period of time, then it loses its influence. The indirect treatment has efficiency in time. The family of microorganisms has different behavior because the green algae and the cyanobacteria are autotrophic and the most bacteria are heterotrophic obtaining food from other organisms. The three data analysis described in the last section show that the microorganisms’ evolution depends on the treatment type, the type of the paints, the samples roughness and the sea water pretreatments. The results show that the biofouling adherence begins after 8h, but the GlidArc effect has a longer time period. In the case of the biofouling presence prior to the test, the GlidArc action has two tasks: to destroy the biofouling layers and to protect the surface for a long time period.

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