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Monitoring ecotoxicological and Environmental Testing Using with Biosurfactants Evaluation of Zone Water Quality Port

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ABSTRACT

Biosurfactants can be used in environmental pollution control systems for oil spill and its derivatives, since they increase the bioavailability of contaminants by reducing the surface tension of water. The mollusk *Anomalocardia brasiliana* is a species that inhabits areas with little suspended material. The aim was to test the tolerance concentration and the median lethal concentration (LC_{50}) for a period of 96h through survival. The experiment was conducted at UFRPE, and the biosurfactant produced the root *Candida lipolytica*, having an industrial waste as a source of carbon, plus mineral medium. The animals were not fed for 24 hr prior to the experiment. It was performed six treatments (1:10, 1:20, 1:40, 1:80 and 1: 160) in three replicates, with the density of 2.5 individual / Litre and subjected to constant aeration at a temperature of 26 ± 0 5 ° C and salinity 26. It follows that the treatments 1 (1:10) and 2 (1:20) gave a survival 76.18%, however treatments 3 (95.23%) and 4 (95 83%) were higher and treating with 5 to 100%. The LC50 estimated for the biosurfactant tested was 0.222 or 1: 4.5. The compound is lethal to the test organism used in dilutions being suggested further experiments with dilutions from 1: 05 to 1:20, to better estimate of LC50 for the same species. Thus, a better understanding of the lethal concentration in organisms representing different trophic levels brings positive results to optimize the procedures in environmental monitoring in port areas.

Keywords: Biossurfactantes, ecotoxicidade, *Candida lipolytica*

INTRODUCTION

The ecotoxicology aims to study the contamination modalities of pollutants arising from human activities, their mechanisms of action and its effects on the set of living beings (MAGALHÃES:FILHO, 2008). It is based on the biological response of the organism to be subjected to chemical agents may, from that, to assess the damage in the body and can be lethal when it leads to death, or immobilization / loss of swimming ability, and sublethal when it does not take death in the body but causes him damage and probably their offspring (MAGALHÃES;FILHO, 2008). In turn, it is a most important tool for the biomonitoring of marine ecosystems and limnology.

Currently, some resolutions are demanding to be realized ecotoxicological tests for the diagnosis of pollution / contamination, as defined in Resolution CONAMA 430/11, as "methods used to detect and evaluate the ability of a toxic agent causing harmful effects, using bioindicators of large groups of an ecological chain." From this test is measured as defined in CONAMA 430/11 as "Lethal Concentration Median - Median Effective Concentration LC50 or - EC50: is the concentration of effluent that causes acute effect (mortality or immobility) at 50% of organisms in a given period exposure, the test conditions."

Recently, the Brazilian Agency for Techniques-ABNT published on May 24, 2016 the NBR 16456: 2016 - Ecotoxicology Aquatic - short test method embryos bivalves (Mollusca - Bivalvae), with the aim evaluating the toxicity of sea water samples, estuarine, interstitial sediment interface and water, wastewater, elutriate, soluble chemical substances or dispersed in water over embriolarval development bivalve Perna, the first technique ABNT related to bivalve molluscs. However, it may be applied to other organisms of the same order rules by using this as a basis.

In fact, for testing with molluscs, it was necessary to use uniform standards of international organizations. Molluscs have a versatility for application testing because of their life cycle, larval-stage planktonic, juvenile and adult-sessile interaction with the sediment. Allows its use for analysis of various types of pollution as the oil in the sediment disposal and what the consequences of this pollution (MONTE et al.,2015).

The bivalve mollusk *Anomalocardia brasiliana* (GMELIN, 1791) Is popularly known as shellfish, clam. The species inhabits preferably protected coastal areas of action of waves and currents, both in the intertidal range as in the shallow subtidal, which burrows superficially into the substrate (BELÉM et al.,2013; BOEHS & MAGALHÃES, 2004). It is a eurythermal species, eurihalina, filter water and free habit, with preference for sites with little suspended material (NARCHI, 1974). Their establishment in sediment frequent suspension of areas is associated with the fact that their siphons are short and fused with simple tentacles (EL-DEIR, 2009).

This mollusk is very important economic and social in the north coast of Pernambuco, and one of the comerciamente explored along the Brazilian coast (BOEHS et al., 2010). The extraction of this kind is for small-scale subsistence fishing and relevant to much of the source of income of coastal communities in the region (LAVANDER et.al, 2011), which makes an important social value organism.

Because of the peculiarities and difficulties in cultivation, the production comes from the extractive fishing, there is no cultivation registers on a commercial scale for this species, may be attributed to the cost and the time it is taken to the commercial production of this species (BOEHS et al., 2010; LAVANDER et al., 2011). However, some laboratories in Brazil are now trying to accomplish the production of this body for the purpose of performing the restocking in areas in antenriores times, the natural stock was located and now is exhausted.

Second Bagliano (2012), the "Bioindicators can respond to contamination by changes in their physiology or their ability to accumulate elements or substances. The response of each organism is strongly influenced by the physical, chemical and biological environment (temperature, humidity, wind and radiation) as well as the physiological condition, nutritional and structural morphological". Thus, it is essential to choose the bioindicator that really represents what is being evaluated due to its influence to correlate a certain anthropic factor or a natural factor with impressive potential, representing an important tool for assessing the ecological integrity (GODEFROID et al., 2015).

Thus, the molluscs are widely used as biomarkers because they are filter feeders and have the ability to filter 19-50 L per minute and feed particles in the water suspension, characterizing the animal with little or no selective ability (COVA et al., 2015; SANDE et al., 2010), facilitating accumulate a variety of bacteria, parasites and heavy metals. Thus, the larger become the accumulator means pollutant (SANDE et al., 2010), It is used for environmental monitoring to be real representatives of the effects of pollution sources (CAO; WANG;. 2016). According Bangliano (2012), the use of bivalve mollusks are best used for ease of location and capture, and the susceptibility to change and may be climate, the water pH, temperature, power, among other factors.

A large pollution source for the environment is the influence of domestic sewage released in nature, which can cause damage to the environment in which it was dumped and the species that inhabit the place, causing risks to human health and interferes with the resistance of the molluscs (DOI et al., 2015). In turn, becomes common bivalve molluscs contaminated by total and fecal coliforms in areas where there is the presence of polluting source. Thus, they become potential vectors for the entry of pathogens in the intestinal tract of man with the possibility of causing disease. It is a mystification that only oysters are vectors of pathogens, however, any bivalves has the same capacity if ingested in nature.

The relevance of monitoring about molluscs in contaminated areas is related there are other dynamics involved. By accumulating pollutants, favoring Biomagnification process, ie the contaminant transfer to higher trophic levels, with tendency to increase the concentration thus may cause harm to human health (FRANCO et al., 2014).

Currently, the fact that the surfactants are produced from chemical oil products has caused consumers to seek alternative compounds obtained from microorganisms existing or natural products generally treated as biosurfactants. This product can be generated from wastes and industrial by products used in various forms, having high potential for industrial application and diversity characteristics, biodegradability, low toxicity and biocompatibility (MORAIS & ABUD, 2012).

However, they have the same property of chemical surfactants to reduce the surface and interfacial tension, both in aqueous solutions and in mixtures of hydrocarbons may also act stabilizing emulsions, so that there is an increase in solubility and availability of hydrophobic contaminants, maximizing the potential for biodegradation (CRUZ, 2012; MELO, 2011) and with the advantage of causing less damage to the environment.

The biosurfactant used was produced base *Candida lipolyticae,* having an industrial waste as a carbon source with mineral medium addition. The use of microorganisms in the product formation is due to the advantage of the use of carbon sources such as power generator, providing increased their microbial community. It can be used different kinds of microorganism such as fungus, bacterium, yeast. Incidentally, according to the biological component to be used, there will be difference in their molecular structure and action engine that its surface to be used, for example, in water interface (SANTOS et al., 2016).

Even with numerous benefits of its use, it is not indicated in the field of food industry at risk of contamination due to potential pathogenic (SANTOS et al., 2016). The use of microorganisms as low-cost substrate results in a cost reduction, because this stage is responsible for 50% of the cost of production (RUFINO et al., 2014).

Due to its properties, the biosurfactants can be used to reduce some damage with high degree of toxicity for example, in case of an oil spill, causing oil spills that reach the fauna and marine flora, with the poisoning of fish, molluscs and other animals that live in this environment may pose additional danger to humans through the food chain (DAMAS, 2000; CRUZ, 2012). The clam *A. Brasiliana,* to be a sessile species, it is more susceptible to contamination because of their reduced mobility and their filtrador habit.

In this context, the *A. brasiliana* was used as the organism test due to its filtering capacity, in order to assess their survival when exposed to a biosurfactant compound and determining the median lethal concentration (CL_{50}) this species bioassays 96 h. Thus, a better understanding of the lethal concentration in organisms representing different trophic levels brings positive results to optimize the procedures in environmental monitoring in port areas.

MATERIALS AND METHODS

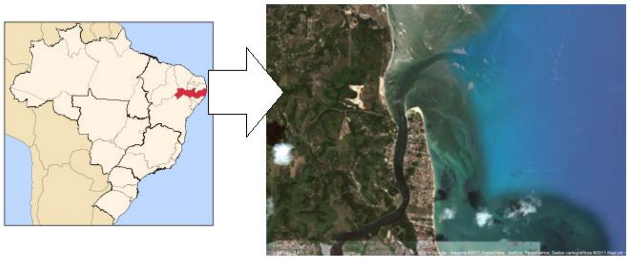
The tests were performed in the quarantine room the Sustainable Mariculture Laboratory (LAMARSU) Department of Fisheries and Aquaculture (DEPAq) University Federal Rural of Pernambuco (UFRPE), in Recife, PE.

The animals used in the tests were collected at various points on the coast of Mangue Seco Beach (07°50'16"S - 034°50'43"W), Nova Cruz, county the Igarassu, Pernambuco (Figura 1). After collect, they were transported to the laboratory and maintained in glass fiber boxes, with volume of 400 L with medium containing seawater salinity 26 and temperature 26±0,5°C, under constant aeration, and fed daily with a mixture of algal *Chaetoceros gracilis* and *Navicula sp.*, in a concentration of 300 x 10^4 cell/mL, for a minimum of ten days, before the experiment.

The microalgae were used as only means of food for the exemplary being produced by means of Conwey culture, vitamin B complex and sodium metasilicate, and submitted to a photoperiod 24 h light, intensity the light of 4000 Lux.

Before performing each test, the specimens were transferred into a tank of 100 liters acclimated to a salinity of 26 and kept without food for 24 hours, for cleaning the digestive tract of animals, thus avoiding interference waste during the experiments with the toxic agent.

Figure 1 - Location of Mangue Seco Beach, on the coast north of Pernambuco, where copies of brasiliana Anomalocardia were collected. Source: Google Earth.

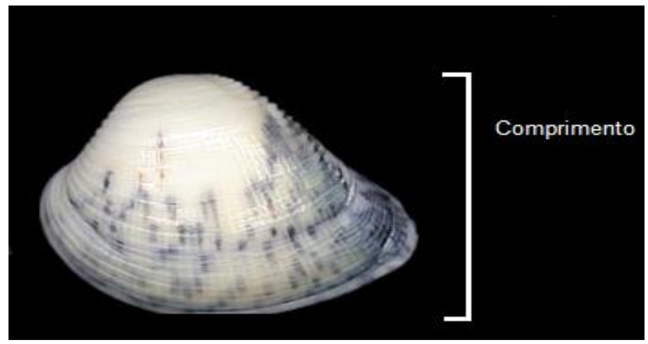


For the realization of the acute toxicity tests 96 hours, glass beakers were employed with a volume of 2.0 liters, filled with the test solution, variable according to the tested dilution and size of individuals, using water with salinity 26, kept under constant aeration, in a room with temperature average the 26±0,5ºC, and photoperiod natural approximately 12 h light / 12 h dark. The experimental units were covered with plastic film to thereby prevent the entry of any body within the experimental units, as in Figure 3. During the period of experiments, animals were not fed or exchange was performed test solution.

They tested five biosurfactant dilutions R: sea water (1:10, 1:20, 1:40, 1:80 e 1:160) for the tested biosurfactant and a control treatment (only sea water), a total of 6 treatments with three replicates each, making a total of 18 experimental units as in Figure 3. We used a ratio of 1 g tissue per mole liter of the test solution in accordance with the recommendations A.P.H.A. (2005).

To calculate this ratio, a previous sample to determine the relationship between the body weight of the clam and the weight of his flesh was made, according to the average size of copies. Biometrics animals was performed from its dorsal-ventral axis (Figure 2), according to Quayle e Newkirk (1989), with copies presented average length of 18,34 mm, total average weight 10,34 g and weight of soft parts 0,396 g. From the relationship between the length of the shell and the weight of the soft tissues used were an average of 5 specimens in each experimental unit (beaker).

Figure 2 – Exemplary the *Anomalocardia brasiliana*, indicating the position measurement of its length. Source: Santos et al., 2011



The lethality of biosurfactants can be classified on the basis of survival of organisms tested in three categories according to the table below.

Table 1 – Degree of toxicity. Source: Author, 2016

Degree of toxicity	
Survival (S)	Classification
S >75%	High survival
50% < S < 75%	Average survival
S < 50%	Low survival

Data analysis was based on a non-linear regression correlating with exponential fit survival data and dilutions tested. Still using the same method can be determined by the median lethal concentration to 50% of persons employed (LC_{50}), expressed in terms of mean mortality of three replicates for each dilution tested. To evaluate the homogeneity and normality were performed the Barlett tests ($p < 0.05$) and Lilliefors ($p < 0.05$), respectively, but these basic assumptions were not met, so the data were analyzed nonparametric

Kruskal -Wallis (H) ($p < 0.05$) in order to observe possible differences between the medians of concentrations analyzed and subsequent comparisons were tested by analysis of multiple comparisons (Dunn, 1964). All these analysis procedures were performed in R 3.2.1 software.

Figure 3 - Experimental design for bioassays of biosurfactant R employing Anomalocardia brasiliana as test organism. Source: Author, 2016.

RESULTS AND DISCUSSION

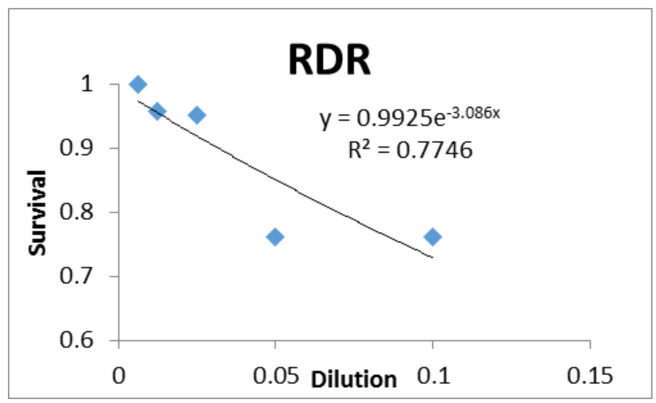
The results of survival experiments in bivalves *A. brasiliana* biosurfactant with different dilutions of R shown in Table 2, and their survival curve shown in Figure 4. After statistical analysis was not possible to detect statistical difference, being equal before the post-hoc Dunn.

The average survival (88,68%) obtained in this work rate this biosurfactant such as low toxicity, it can be observed in the values obtained for individual treatments. The median lethal dilution (LC_{50}) estimated for the biosurfactant tested was 0.222, which corresponds to a dilution of 1: 4.5, which is higher than that obtained by Santos et al. (2013) using the same organism to test the toxicity to a given biosurfactant.

Table 2 - The bivalves survival values *Anomalocardia brasiliana* subjected to different dilutions of biosurfactant R. Source: Author, 2016.

Dilution	Survival
1:10	76,18%[a]
1:20	76,18%[a]
1:40	95,23%[a]
1:80	95,83%[a]
1:160	100%[a]
Control	100%[a]

Figure4 Bioassay survival curve 96h with the bivalve *Anomalocardia brasiliana* subjected to different dilutions of biosurfactant R. Source: Author, 2016.



The results molluscs were more satisfactory compared to the experiment conducted with microcrustacean *Artemia sp.* with the same biosurfactant and the same dilutions made by Santos et al. (2012), in which the best result was in the 1:40 dilution to 83.3%, and the worst observed in 1:10 dilution with 3.3% survival, which can be classicado to this organism the average standard toxicity.

The divergence of results between organisms can be linked to form, because the seafood to be a bivalve mollusk spend less time exposed to the toxic agent, the only form of entry through the trap during the breathing process. However, *Artemia sp.* is exposed throughout the test duration as not have a protective shell, there is probability that the toxic effect is more pronounced because the biosurfactant of particles available in the experimental unit can join the body, emphasizing its toxicity.

Thus, in view of these results, it was observed that the biosurfactants tested showed mixed lethality for bivalve mollusc *Anomalocardia brasiliana*, considering the dilution test between 1:10 and 1: 160, with greater survival in the lower dilutions as expected. Thus, to conclude that the compound has lethal for the organism tested, and suggest the need to carry out new experiments with dilutions between 1:05 and 1:20 to better estimate of LC_{50} for the compound species. Thus, better understanding of the lethal concentration in organisms representing different trophic levels, brings in positive results to improve procedures in environmental monitoring in port areas.

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