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Antifouling Protection of Surfaces Immersed in Marine Environment by Natural Surfactants as Bioactive Contained in Coating Based on Natural Resin

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Surface biofouling of materials immersed in the marine environment is a problem that particularly affects maritime industries and equipment. The biological community present in these environments develops on the immersed surfaces of different structures, causing economic damages to the local facilities. Antifouling coatings based on synthetic chemicals, while being the primary strategy used to combat fouling in the marine industry, are detrimental to other ecosystem beings in addition to target organisms (biofouling). To avoid or reduce this biofouling, a non-biocidal strategy is the application of coatings based on natural surfactants, making them more hydrophobic, imposing it difficult to fix biofouling. In this context, the aim of the present study was to formulate a non-biocidal antifouling coating containing natural surfactants, obtained by fermentation process and/or by chemical modification of residual soybean oil, evaluating its antifouling effect on local marine biofouling. To this end, a natural resin-based matrix containing the natural surfactants was prepared, which was applied to metal panels and after curing the coating, these were followed for immersion tests at the Port of Recife-PE, Brazil for 25 days. After this period, the panels were photographed and analysed for the macrofouling biota present. There was a reduction in biofouling around 30% compared to untreated panel. After immersion tests at the Port of Recife-PE and analysis of the covered area, the panel coated by the matrix + laurate and matrix + hydroxylated acid exhibited the best antifouling activity during the period tested. The results of the embedded area, through the ImageJ software, demonstrated the promising effect of the antifouling coating in the field, evaluated in the treatments in relation to the negative control, evidencing the great biotechnological potential of natural surfactants in the treatment of the biofouling.

1. Introduction

Surface biofouling of materials immersed in the marine environment is a problem that particularly affects marine equipment and industries (Gittens et al., 2013; Gule et al., 2016). The fouling marine biological community defined as biofouling, develops in these submerged artificial substrates, in four phases: generation of a layer of organic molecules; primary colonization by biofilms (viruses, bacteria, fungi and diatoms); unicellular colonization by algal spores; and finally, the fixation of multicellular macrofouling occurs (Donlan, 2002; Gule et al., 2016). In general, for the establishment of biofouling, it is necessary, first, the presence of biofilms (Palanisamy et al., 2017). Microorganisms associated with local organisms produce secondary metabolites and secrete molecules to the environment with attractive signals, initiating the fixation and the larval metamorphosis (Satheesh et al., 2016). These molecules increase the chance of survival and the

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process of fixing and developing larvae of fouling organisms under unfavorable conditions, as well as attract other organisms (Dobretsov, 2010; Maruzzo et al., 2012). Industrial water pipes are highly favorable sites for the development and proliferation of microorganisms. Consequently, the formation of biofilms in equipment and channels is a nearly impossible process to prevent (Cristiani, 2005).

Anti-fouling coatings based on synthetic chemicals is the primary strategy to combat biofouling in the marine industry. Biocides containing TBT (tributyltin), although effective in reducing biofouling, are banned in several countries because they are harmful to other organisms in the marine environment besides fouling organisms (Gittens et al., 2013).

To prevent or reduce biofouling on immersed surfaces, one of the current trends is to change surfaces to make them more hydrophobic. Another strategy is the addition of natural antimicrobial substances (Jagani et al., 2009) in different types of natural or manufactured coatings and coat the surfaces with these products, eliminating or hindering microbial adhesion, once biofilms colonize a surface, they alter the surface properties and facilitate the adhesion process by another biofouling (Gittens et al., 2013; Damodaran and Murthy, 2016; Doghri et al., 2011).

Surfactants are chemical compounds that have amphipathic properties that reduce the surface and interfacial tensions of liquids. Such characteristics allow them to interact with different molecules of different affinities and with that they are used in several applications (Sivapathasekaran and Sen, 2017). Such compounds have a predilection for interfaces of dissimilar polarities and are soluble in both organic (non-polar) and aqueous (polar) solvents (Almeida et al., 2016). They can be obtained by petroleum derivatives (most surfactants available on the market), by vegetables and micro-organisms (Santos et al., 2016).

Due to their toxic character, synthetic surfactants have been gradually replaced by natural surfactants (biosurfactants) in several applications in last years (Dey et al., 2015). These surface-active molecules of biological origin have several advantages over synthetic surfactants such as higher biodegradability, low toxicity, better environmental compatibility, better activity and resistance in extreme conditions of temperature, pH and salinity, and higher selectivity for metals and organic compounds and can be synthesized from renewable feedstocks (Pinto et al., 2018; Santos et al., 2016). In addition to their antibacterial, antiviral and antifungal activities, these compounds have also proved to be good inhibitors of microbial adhesion and biofilm formation (Araujo et al., 2016; Silva et al., 2018; Santos et al., 2016).

In some studies, it was observed that a mixture of natural surfactants (palmitic, stearic, oleic and linoleic acids) was able to inhibit AI-2 activity, disturbing the microbial quorum sensing, thus indicating that these surface-active molecules inhibit biofilm formation and, in turn, inhibit the larval establishment (Fusetani, 2011; Goto et al., 1992; Soni et al 2008).

Starting from the analysis that biofouling is formed on surfaces and microorganisms are the pioneers in these formations contributing to the emergence of another biofouling. When using surface modifying elements, it is assumed that there is a change in the chain of adhesion events and consequent formation of biofouling (Parsons et al., 2012; Nascimento et al., 2011).

Therefore, the present work had the objective of formulating and evaluating coatings containing non-toxic natural surfactants of antimicrobial action already evaluated in recent studies by Silva et al. (2018) as antifouling agents, with potential for application as a coating on submerged surfaces in sea water with the presence of fouling organisms.

2. Material and methods

Obtaining the natural surfactants from the residual soybean oil (*Glycine max* (L.))

All steps for the synthesis of surfactants using the fatty acid obtained from soybean oil (Glycine max (L.)) as the main molecule were performed according to the methodology of Silva et al. 2018. The following substances were produced: 9,10-dihydroxy octadecanoic acid (hydroxylated oleic acid), sodium 9,10-dihydroxy octadecanoate (sodium salt of hydroxylated oleic acid), 2,3-dihydroxy- (glyceryl oleate), 2,3-dihydroxypropanoyl dodecanoate (glyceryl laurate) and 2,3-dihydroxypropanoyl 9,10-dihydroxy octadecanoate (hydroxylated glyceryl oleate), by the chemical modification of soybean oil (*Glycine max* (L.)) by neutralization reactions, esterification of glycerol and hydroxylation reactions by epoxidation, using suitable catalyst. The rhamnolipid was obtained from the bacterium *Pseudomonas cepacia* (Soares da Silva et al., 2017) and the soybean lecithin acquired from Nutryervas do Brasil LTDA.

Preparation of the soluble resin matrix

This matrix was used for the dispersion of coatings with antifouling property. The composition used to obtain the coating was adapted from (Acevedo et al., 2013) and expressed as a percentage by weight: 27% natural resin, 6% oleic acid, 20% xylene, 20% white spirit, 16.2% zinc oxide and 10% 8% calcium carbonate. The

dispersion of all components was performed on a mechanical agitator (Tecnal LTDA, Brazil) at 2000 rpm for 50 min.

Incorporation of substances (natural surfactants) obtained from soybean oil (*Glycine max* (L.))

After individual extraction of the elements mentioned in item 2.1, they were stored appropriately and later incorporated into the 10% (w / w) natural resin soluble matrix. In the table below the test compositions of each coating.

Table 1: Mixtures formulated with natural surfactants to obtain antifouling

Mixture	Composition
Control	Matrix without active
Mixture 1	Matrix + glycerol / lauric acid oligomer surfactant
Mixture 2	Matrix + glyceryl laurate
Mixture 3	Matrix + hydroxylated oleic acid
Mixture 4	Matrix + biosurfactant
Mixture 5	Matrix + soybean lecithin
Mixture 6	Matrix + sodium salt of hydroxylated oleic acid
Mixture 7	Matrix + hydroxylated glyceryl oleate

Fields studies by immersion tests

Metal panels (1mm x 100mm x 200mm) were washed with analytical grade acetone to degrease the surface. Then, one coat of each formulated matrix was applied to the cleaned panels using a one-inch universal brush. After 1 to 2 hours, for solvent evaporation, a second coat was applied. The panels remained dry for up to a week at room temperature and protected from the sun. They were numbered for identification and drilled (3mm diameter opening) to assist in fixing to the rail holder.

The in-situ immersion tests were carried out in the Port of Recife S.A., Recife / PE. At this site, the metal panels containing the experimental matrices were immersed in an area with high occurrence of barnacles and other fouling organisms. The plaques were checked initially after 7 days and after 25 days of immersion, in the above area. Photos were taken from all the panels, however, only the images of the panels with less biofouling passed through analysis of the surface covered with the help of the software image editor "ImageJ". The area covered by the fouling macro-organisms on the panels was calculated as the percentage of the area occupied by these organisms in relation to the total area examined, normalized with respect to the covered area relative to the control.

3. Results and discussions

In Figure 1 (Figures 1A - 1B), we have the results obtained in field tests, after 7 days (A) and 25 days (B) of the panels immersed in the Port of Recife. There was a biofouling delay evidenced by the difference in coverage on the panels, as shown in figure 1.

In Figure 1 (B) it was observed that panels 2, 5, 6, 9 and 10 were completely embedded with macrofouling after 25 days of testing. On the other hand, panels 1, 3, 4 and 7 presented very promising results, as evidenced by the delay observed in the scale process. Panel 8, relative to the matrix containing the sodium salt of hydroxylated oleic acid, was detached from the rail, and it was not possible to analyze the antifouling potential of this surfactant until the end of the experiment.

Figure 2 presents the analysis of the percentages of covered area of the most representative panels of the different results obtained in the field, calculated by ImageJ software after 25 days. The measurement by the application indicated that panels 3 (62.444%) and 4 (72.336%) showed incrustation delay in comparison to the matrix panel (82.700%) and panels 10 (98.187%) completely covered.

The surface area of panels coverage was measured by converting the images to binary scale using ImageJ software and adjusting the threshold to differentiate precisely between areas with and without macrofouling (Figure 3).

In general, panels 1,3 and 4 (matrix, matrix + glyceryl laurate, matrix + hydroxylated oleic acid, respectively) were found to have the best results. There was a reduction in biofouling around 30% compared to untreated panel. To avoid biofouling, two approaches may be employed: (I) to prevent the development of the early stages of biofouling by employing bioactive in a non-lethal manner for the environment, and (II) formulating and applying larval fixation inhibitory coatings, making the surface not suitable for the development of biofouling (Bressy et al., 2014; Jerabek et al., 2016).

In this work, the two strategies were simultaneously applied, and as a result, the matrix + glyceryl laurate (panel 3) and the matrix + hydroxylated oleic acid (panel 4) presented the best inhibition potentials for biofouling development.
Acevedo et al. (2013) also evaluated a natural matrix, however, added to extracts of marine animals (sponge and cucumber) during 90 days of immersion, obtaining anti-biofouling activity.

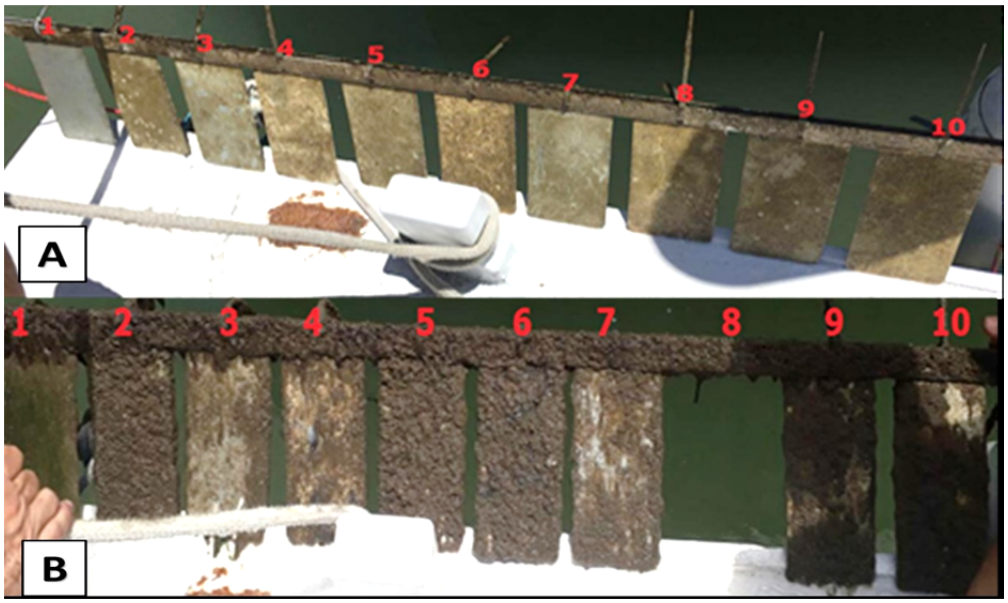

Figure 1: Panels after 7 days (A) and 25 days (B) immersed in the Port of Recife. 1: Matrix (without natural surfactants); 2: Matrix + glycerol oligomer surfactant; 3: Matrix + glyceryl laurate; 4: Matrix + hydroxylated oleic acid; 5: Matrix + glycolipid; 6: Natural resin; 7: Matrix + lecithin; 8: Matrix + hydroxylated sodium oleic acid; 9: Matrix + hydroxylated glyceryl oleate; 10: No coating and / or treatment.

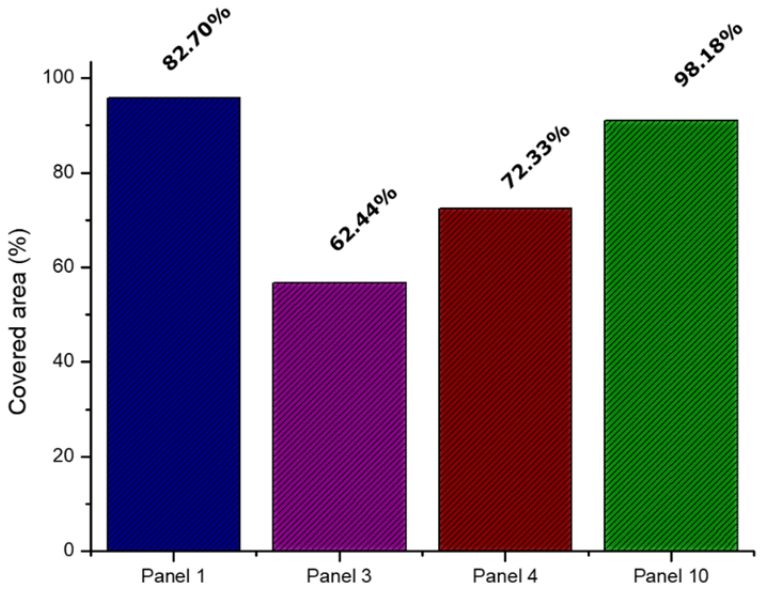

Figure 2: Percentages of the area covered by macrofouling after 25 immersion in the Port of Recife, Recife-PE, calculated by ImageJ software.

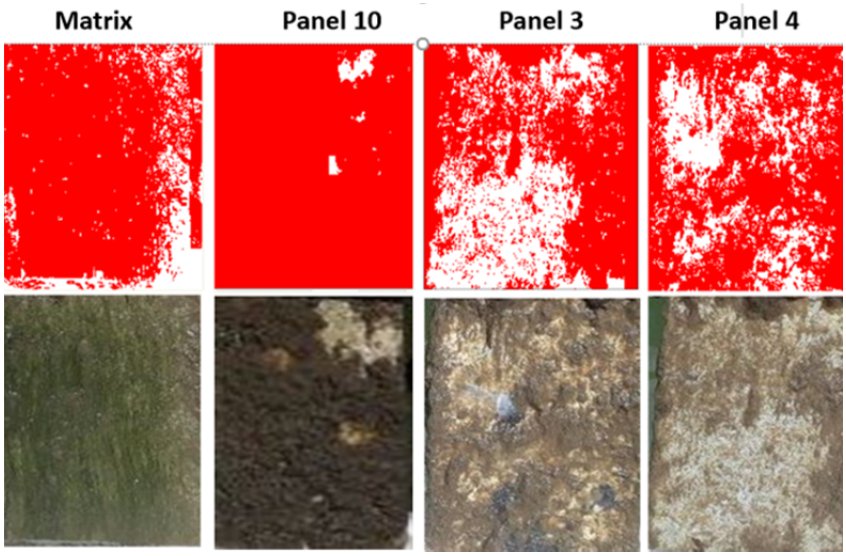

Figure 3: Analysis of the surface area covered by the ImageJ software of the panels after 25 days submerged in the Port of Recife, Recife - PE. Top images: treatment for analysis of the area covered by ImageJ software. Images below: corresponding real panels.

The widespread use of toxic biocides in antifouling paints has introduced high levels of contamination into the environment and raised concerns about its toxic effects on marine communities. Natural product antifouling is one of the most promising alternatives to replace the used antifouling agents which are both toxic and non-biodegradable. Thus, when using new environmentally-friendly products, high levels of contamination in the environment are avoided. As in the case of the present research, the use of residual materials to obtain substances with special functions was used.

4. Conclusions

The compositions tested from the Matrix, Matrix + laurate and Matrix + hydroxylated acid showed very promising antifouling activity during the period tested. The results demonstrated that it is possible to obtain natural surfactants with antifouling activity from residual sources. However, in order to obtain a more effective antifouling protection response, the concentration of the natural surfactants in the matrix must be increased, as well as time-to-time release of the compounds in the environment as the behavior of these substances present in the composition of the matrix and the various synthesized surfactants act differently depending on the polarity of the fluids or materials where they are placed.

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References

Acevedo, M.S., Puentes, C., Carreño, K., León, J.G., Stupak, M., García, M., Pérez, M., Blustein, G., 2013. Antifouling paints based on marine natural products from Colombian Caribbean. Int Biodeterior Biodegradation 83, 97–104. https://doi.org/10.1016/j.ibiod.2013.05.002

Almeida, D.G., Soares da Silva, R. de C.F., Luna, J.M., Rufino, R.D., Santos, V.A., Banat, I.M., Sarubbo, L.A., 2016. Biosurfactants: Promising Molecules for Petroleum Biotechnology Advances. Front Microbiol 7, 1718. https://doi.org/10.3389/fmicb.2016.01718

Araujo, L.V. de, Guimarães, C.R., Marquita, R.L. da S., Santiago, V.M.J., de Souza, M.P., Nitschke, M., Freire, D.M.G., 2016. Rhamnolipid and surfactin: Anti-adhesion/antibiofilm and antimicrobial effects. Food Control 63, 171–178. https://doi.org/10.1016/J.FOODCONT.2015.11.036

Bressy, Christine; Lejars, M., 2014. Marine Fouling : An Overview Marine Fouling An Overview. J Ocean Technol 9.

Cristiani, P., 2005. Solutions to fouling in power station condensers. Appl Therm Eng 25, 2630–2640. https://doi.org/10.1016/j.applthermaleng.2004.11.029

Damodaran, V.B., Murthy, N.S., 2016. Bio-inspired strategies for designing antifouling biomaterials. Biomater Res 20, 18. https://doi.org/10.1186/s40824-016-0064-4

Dey, S., Malik, S., Ghosh, A., Saha, R., Saha, B., 2015. A review on natural surfactants. RSC Adv 5, 65757–65767. https://doi.org/10.1039/c5ra11101c

do Nascimento, L.A.S., Tito, L.M.Z., Angélica, R.S., da Costa, C.E.F., Zamian, J.R., da Rocha Filho, G.N., 2011. Esterification of oleic acid over solid acid catalysts prepared from Amazon flint kaolin. Appl Catal B Environ 101, 495–503. https://doi.org/10.1016/j.apcatb.2010.10.021

Dobretsov, S., 2010. Marine Biofilms. Biofouling 123–136. https://doi.org/10.1002/9781444315462.ch9

Doghri, I., Rodrigues, S., Bazire, A., Dufour, A., Akbar, D., Sopena, V., Sablé, S., Lanneluc, I., 2011. Marine bacteria from the French Atlantic coast displaying high forming-biofilm abilities and different biofilm 3D architectures. https://doi.org/10.1186/s12866-015-0568-4

Donlan R.M., 2002, Biofilms: Microbial Life on Surfaces, Emerging Infectious Diseases, 8, 881–890.

Fusetani, N., 2011. Antifouling marine natural products. Nat Prod Rep 28, 400–410. https://doi.org/10.1039/C0NP00034E

Gittens, J.E., Smith, T.J., Suleiman, R., Akid, R., 2013. Current and emerging environmentally-friendly systems for fouling control in the marine environment. Biotechnol Adv 31, 1738–1753. https://doi.org/10.1016/j.biotechadv.2013.09.002

Goto, R., Kado, R., Muramoto, K., Kamiya, H., 1992. Fatty acids as antifoulants in a marine sponge. Biofouling 6, 61–68. https://doi.org/10.1080/08927019209386210

Gule, N.P., Begum, N.M., Klumperman, B., 2016. Advances in biofouling mitigation: A review. Crit Rev Environ Sci Technol 46, 535–555. https://doi.org/10.1080/10643389.2015.1114444

Jagani, S., Chelikani, R., Kim, D.-S., 2009. Effects of phenol and natural phenolic compounds on biofilm formation by Pseudomonas aeruginosa. Biofouling 25, 321–324. https://doi.org/10.1080/08927010802660854

Jerabek, A.S., Wall, K.R., Stallings, C.D., 2016. A practical application of reduced-copper antifouling paint in marine biological research. PeerJ 4, e2213. https://doi.org/10.7717/peerj.2213

Maruzzo, D., Aldred, N., Clare, A.S., Høeg, J.T., 2012. Metamorphosis in the cirripede Crustacean Balanus amphitrite. PLoS One 7, 1–8. https://doi.org/10.1371/journal.pone.0037408

Palanisamy, S.K., Rajendran, N.M., Marino, A., 2017. Natural Products Diversity of Marine Ascidians (Tunicates; Ascidiacea) and Successful Drugs in Clinical Development. Nat Products Bioprospect 7, 1–111. https://doi.org/10.1007/s13659-016-0115-5

Parsons, J.B., Yao, J., Frank, M.W., Jackson, P., Rock, C.O., 2012. Membrane disruption by antimicrobial fatty acids releases low-molecular-weight proteins from staphylococcus aureus. J Bacteriol 194, 5294–5304. https://doi.org/10.1128/JB.00743-12

Pinto, M.I.S., Ribeiro, B.G., Guerra, J.M.C., Rufino, R.D., Sarubbo, L.A., Santos, V.A., Luna, J.M., 2018. Production in bioreactor, toxicity and stability of a low-cost biosurfactant. Chem Eng Trans 64, 1–6. https://doi.org/10.3303/CET1864100

Santos, D.K.F., Rufino, R.D., Luna, J.M., Santos, V.A., Sarubbo, L.A., 2016. Biosurfactants: Multifunctional Biomolecules of the 21st Century. Int J Mol Sci 17, 401. https://doi.org/10.3390/ijms17030401

Satheesh, S., Ba-akdah, M.A., Al-Sofyani, A.A., 2016. Natural antifouling compound production by microbes associated with marine macroorganisms — A review. Electron J Biotechnol 21, 26–35. https://doi.org/10.1016/j.ejbt.2016.02.002

Silva, M.D.G.C., Almeida, D.G., Soares da Silva, R.D.C.F., Meira, H.M., Almeida, F.G., Fernandes, M.L.B., Silva, A.K.P., Santos, V.A., Sarubbo, L.A., 2018. Chemically modified natural substances screening for biofilms inhibition and biofouling control. Chem Eng Trans 64. https://doi.org/10.3303/CET1864110

Sivapathasekaran, C., Sen, R., 2017. Origin, properties, production and purification of microbial surfactants as molecules with immense commercial potential. Tenside, Surfactants, Deterg 54, 92–104. https://doi.org/10.3139/113.110482

Soares da Silva, R. de C.F., Almeida, D.G., Meira, H.M., Silva, E.J., Farias, B.B., Rufino, R.D., Luna, J.M., Sarubbo, L.A., 2017. Production and characterization of a new biosurfactant from Pseudomonas cepacia grown in low-cost fermentative medium and its application in the oil industry. Biocatal Agric Biotechnol. https://doi.org/10.1016/J.BCAB.2017.09.004

Soni K.A., Jesudhasan P., Cepeda M., Widmer K., Jayaprakasha G.K., Patil B.S., Hume M.E., Pillai S.D., 2008, Identification of ground beef-derived fatty acid inhibitors of autoinducer-2-based cell signalling, Journal of Food Protection, 71, 134–138.