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Culture of Microalgae with Ultrafiltered Seawater: A Feasibility Study

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Abstract

The culture of microalgae is important for the production and maintenance of bivalves. One of the major challenges is to maintain the reliability of microalgae forages over the long term. The aim of this work is to use Ultrafiltered (UF) seawater to cultivate them. Thus, cultures in a volume of 300 L of 2 species of microalgae Tetraselmis and T-isochrysis, were monitored in UF water (membrane pore size: 20 nm) and in sea water usually used on the Ifremer mollusk experimental platform of Bouin (France) (Prefiltration, 3 filtrations and 2 UV). The major result is the securing of microlagae cultures with the absence of parasites in all cultures supplied with ultrafiltered water, unlike analyses of the various control cultures. In the case of T-isochrysis, 3 cultures out of 4 resulted in higher microalgae concentrations, up to 30%, in ultrafiltered water thus bringing a benefit on the algal density. These conclusions and the ease of recovering water (linked to the reduction in treatment stages) allowed a transfer of technology. In fact the 300 L cultures hitherto carried out on the experimental platform are now produced in ultrafiltered water since early 2019.

Keywords: Microalgae; Ultrafiltration; Water Purification; Shellfish Production.

1. Introduction

Microalgae production is intertwined with shellfish production structures under controlled conditions. Different strains can be cultivated, the species commonly used in hatcheries must be/have (i) a size and shape suitable for ingestion and digestion by oysters at their different stages of life, (ii) a good nutritional quality, (iii) toxin-free, (iv) cultivable on a large scale and (v) resistant to fluctuations in temperature, light and nutrients (Guedes and Xavier Malcata 2012 [1]; Helm 2004 [2]; Wallace *et al.* 2008 [3]). Among the most cultivated species, two flagellate species: Tetraselmis suecica and T-Isochrysis lutea have been studied. Their nutritional qualities make them the species mainly cultivated under controlled conditions in shellfish hatcheries to feed oysters from fecundation to adult stages (Helm 2004 [2]; Wallace *et al.* 2008 [3]; Brown *et al.* 1997 [4]; Wikfors *et al.* 1996 [5]). One of the challenges is to maintain the reliability of the microalgae culture methods during a long period [1]. One of the problems for these cultures is the great dependence on water quality [6]. Vibrio species, including Vibrio parahaemolyticus, V. vulnificus, and V. cholerae, are common pathogens causing seafood-borne illnesses worldwide. Mok *et al.* (2019) [7] monitored the distributions of pathogenic Vibrio strains in seawater and bivalves and they determined the features of virulence and

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antibiotic resistance in V. parahaemolyticus isolates. More than half of the isolates were resistant to at least three antimicrobials, in particular, three antibiotics. The consumption of raw seafood, including oysters, is common in Korea; therefore, to ensure seafood safety, continuous monitoring of Vibrio strains, as well as their virulence and antimicrobial resistance, is necessary in marine food sources and more especially in the case of microalgae culture. Lumbessy (2019) [8] say that semi-sterile culture is an initial maintenance stage that is essential to be optimized to get sterile seaweed explants. For example, they showed that hydroponic media have the highest average weight and survival rate of G. salicornia (48.76 g and 99.75%). In the case of production of high purity water electrodeionization (EDI) Wardani *et al.* (2017) [9] and reverse osmosis (RO) Greenlee *et al.* (2008) [10] is the most common method to produce high purity water used for microelectronic and pharmaceutical industries for low and large quantity respectively but high purity water without organic matter and salt is obtained. At the opposite, ultrafiltration seem appears as the best process to stop bacteria and virus without modification of salts and organic compound concentrations. In the context of microalgae protection, the first studies Ferguson *et al.* (1984) [11] on 10 liters show that filtration with a cutoff threshold of 3 microns is not enough to stop bacteriological pollution while 0.2 micron appears sufficient. It should be noted that this molecular weight cut off relates to microfiltration and lets viruses pass through. Similarly, Huq *et al.* (1996) [12], in the specific case of V. cholerae, have shown the possibility of V. cholerae was enumerated before and after filtration to evaluate the efficiency of the filtration procedure. The results obtained indicate that 99% of V. cholerae, i.e., those cells attached to plankton, were removed from the water samples. These results were obtained with a filter constructed from either nylon net and one of several different types of sari material but the pore size is not determined. Moreover the ultrafiltration has been studied during harvesting of microalgae in a lab- and a full-scale test [13]. The performances of both scales are compared and analyzed to provide an understanding of several aspects which affect the yield produced from lab and actual conditions. UF exhibits several advantages, such as simple piping and connection, single pump for filtration and backwashing, and smaller footprint.

So, in order to avoid contamination during the algae culture growing, leading to slower density cultures or premature cell death, and to protect the shellfish from possible contamination by pathogens during feeding, the water used for phytoplankton growing must be disinfected. This study is positioned in the context of microalgae culture with the objective of purify seawater by ultrafiltration in order to grow microalgae under the best conditions. Most of time, the conventional treatments (about 6 treatment steps) used in shellfish farming (in this work Ifremer – Bouin, France) is a serie of filtration stages and disinfection by UV radiation. First, decantation/filtration steps at 10-100 microns depending on the media used and UV irradiation are used. An oxygenation step can be carried out before thinner filtration steps from 5 to 0.22 microns. In the case of the ultrafiltered water, the water after decantation is pretreated by sand filtration before direct purification by membrane. These two chains of 6 and 3 unit operations will be compared for the culture of microalgae. The novelty of this paper is to study ultrafiltration for seawater purification to produce microalgae cultures in order to validate the use of this process to reduce the number of treatments and ideally to improve the culture conditions.

2. Materiel and Methods

The tests focused on cultures in cylindrical tanks allowing the production of a culture volume of 300 L. These tanks are previously cleaned and disinfected to eliminate any risk of microalgae contamination. The cultures are carried out in real hatchery conditions, in semi-continuous mode: a volume of microalgae (between 50 and 200 L) is subtracted daily to feed the oyster breedings then the cultures are supplemented to 300 L with purified water. Four cylindrical tanks (4× 300 L) are used for this study focusing on the 2 targeted microalgae species. They are each cultivated at the same time, either in ultrafiltered water or in purified water according to the treatment chain described in the previous part. The microalgae concentrations were monitored daily in the 4 tanks by spectrophotometry. A volume of 1 mL was used to measure the absorbance value at 654 nm in a spectrophotometer Thermo Scientific Evolution 220 allowing the determination of the cell concentration (correlation between absorbance and cell concentration were previously determined for each microalgae). To complete these concentration measurements, monitoring of physico-chemical parameters was also carried out on the microalgae cultures and on the water supplying the culture tanks.

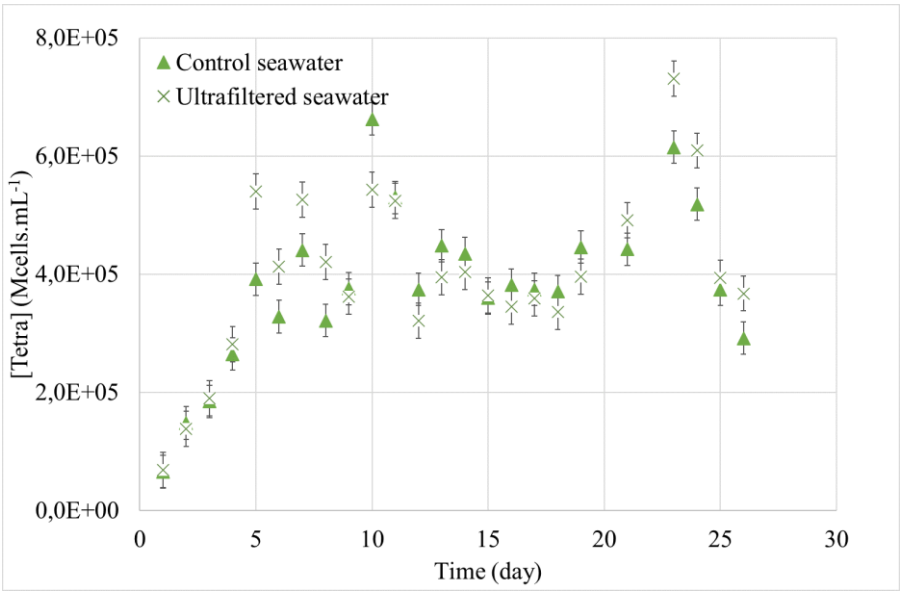
Observations under the microscope were also carried out to visually compare the microalgae qualities according to the size, shape and mobility of the cells, the presence of predators, etc. Predators resistant to different treatments and thus threatening the culture can be (i) phytoplankton or other invasive microalgae species and / or (ii) zooplankton, such as ciliates, consumers of microalgae or (iii) bacteria. The objectives of these analyzes are to (a) control the growth of the microalgae strains in the different waters, (b) ensure the well-conditions of the cultures by following the "key parameters", particularly pH and dissolved O_2, and (c) verify the absence of predators. These cultures were carried out several times, over a period of 10 days for *Isochrysis* and 3 weeks for *Tetraselmis*, which are the maximum durations of these cultures to obtain a production stability. The ultrafiltration membrane used for this study were Aquasource hollow fibers in polyethersulfone with 7 channels of a 0.9 mm inside diameter. Their pore size was 0.02

µm and initial seawater permeability 800 $L.h^{-1}.m^{-2}.bar^{-1}$. The membrane module area of 8 m² that was able to treat 20 $m^3.d^{-1}$ was already described by Cordier *et al.* (2018a, b; 2019) [14-16].

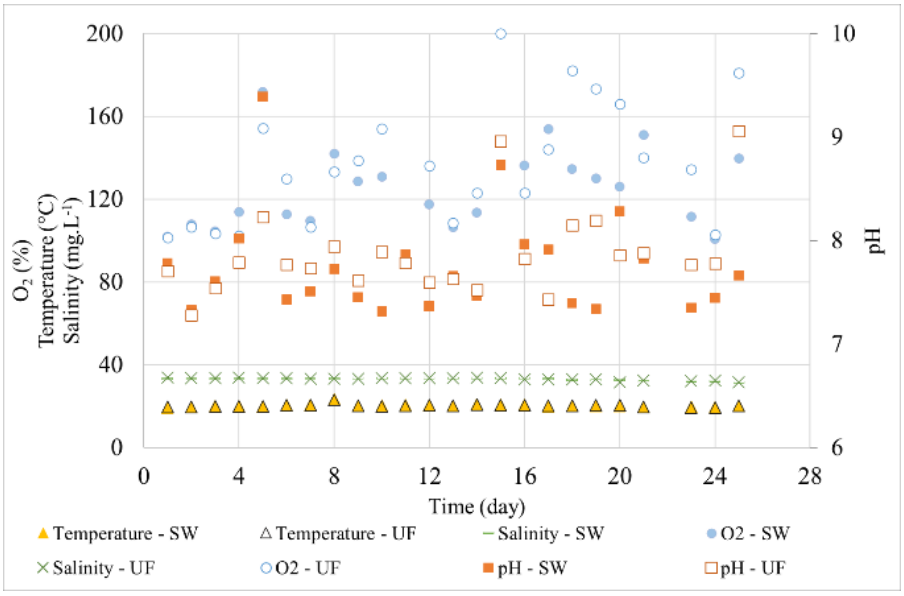
The tests were all performed in dead end filtration and the permeate was recovered in a buffer tank in order to perform backwashing. Three membrane cleanings were automatically carried out by the pilot to eliminate fouling: classical backwashes (CB), air backwashes (AB) which consists in a previous air injection in the membrane before classical backwashing (CB), and chemical cleaning. To follow hydraulic performances, Lp and TMP, respectively membrane permeability and transmembrane pressure, were calculated and recorded continuously every minute. All the results are expressed taking into account the variation of temperature. Filtration conditions constant permeate flux (60 $L.h^{-1}.m^{-2}$) and filtration time (60 min), were selected according to the literature Guilbaud *et al.* (2019, 2018) [17, 18] and previous studies Cordier *et al.* (2018a, 2018b) [14, 15].

3. Results and Discussion

The daily measurement of concentrations obtained for the two cultures is presented in Figure 1a. These results highlight a similar cellular concentration of *Tetraselmis* in the two waters over the duration of the culture (5 weeks). The reproducibility of these results is validated on several tests. This result is also confirmed by the monitoring of the physico-chemical parameters of the waters, similar for these two tests (Figure 1b).



(a)



(b)

Figure 1. Monitoring of Tetraselmis concentrations (a) and physico-chemical parameters (b) - SW: seawater treated with common processes (filtrations + UV) and UF: ultrafiltered seawater

Similar cell concentrations were obtained in the control seawter and in the ultrafiltered seawater on a 10 days period. However, observations under the microscope showed that ultrafiltered water offers higher protection of cultures against predators. Indeed, after 1 month of culture, ciliates were observed in the culture performed in the control seawater, although it was never detected with ultrafiltered water. Figure 2 show some kinds of parasite which could be observed in the control culture. This higher bio-securisation of the microalgae culture with ultrafiltered seawater was confirmed on several tests.

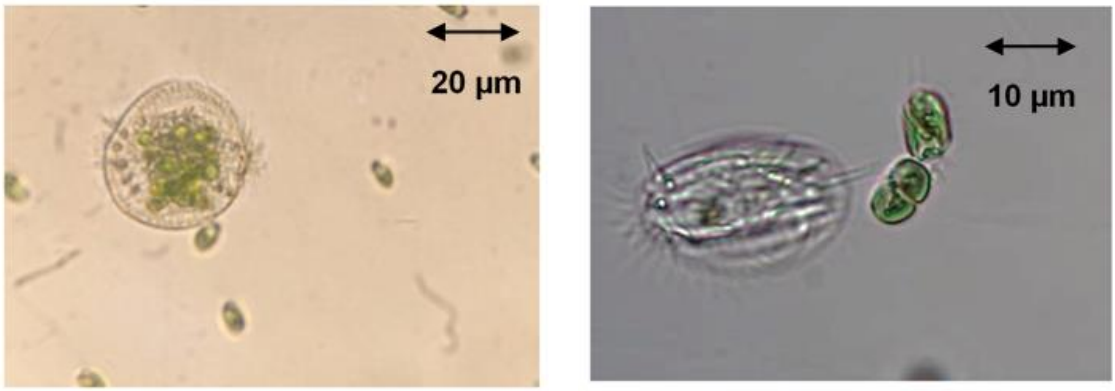
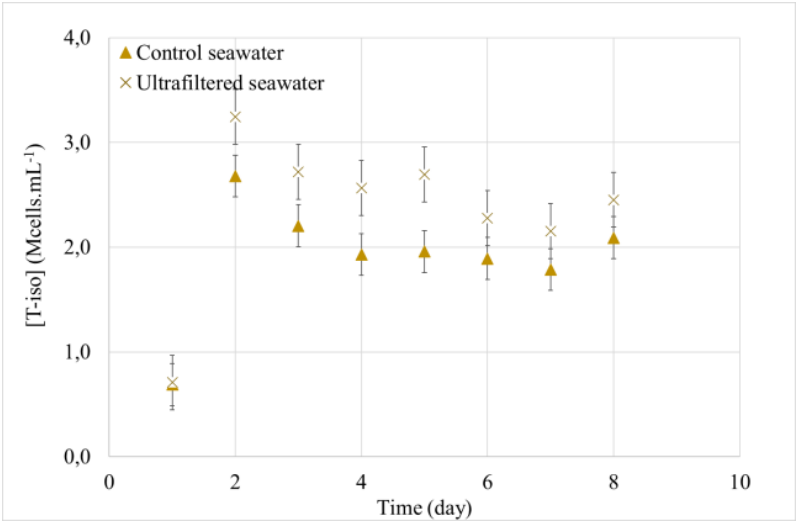
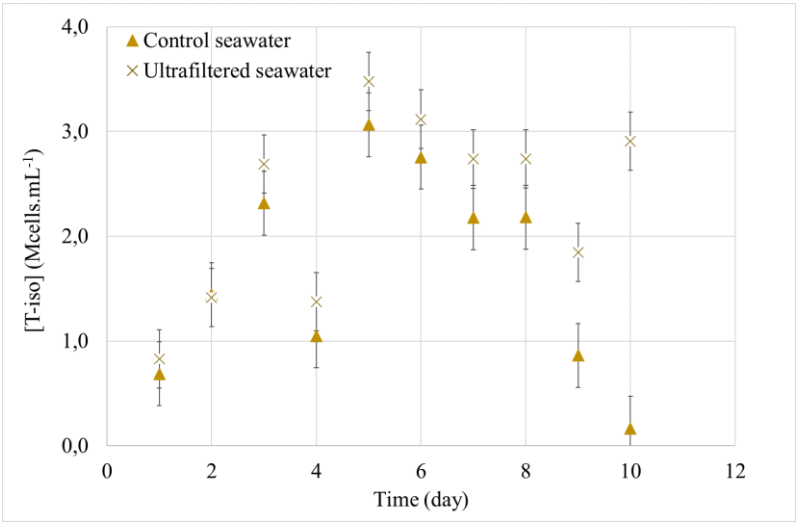


Figure 2. Predators (ciliates) observed in a control Tetraselmis culture in seawater

The monitoring of *T-lsochrysis* concentration is presented in Figure 3.



(a)



(b)

Figure 3. Monitoring of T-Isochrysis concentrations

Unlike *Tetraselmis* cultures, the results obtained for *T-Isochrysis* allow to highlight a significant benefit of UF water on the cell concentration obtained. Indeed, a higher cell concentration is always obtained in UF water varying from 6 to 30% during the different tests. If the quality of water produced is suitable for this application, it, also, offers better culture security compared to seawater produced with a conventional treatment. Ultrafiltration is an integrative alternative water treatment to provide a better protection of microalgae cultures against predators, higher growth performances in the case of the *Isochrysis* microalgae and a decrease of water treatment units to be implemented. In this study, only one step is necessary after decantation and prefiltration, against 4 (UV, 5 µm filtration, UV and 0.22 µm filtration) in the conventional process. Following these experiments, the use of ultrafiltered water is validated for all the 300 L container cultures for *Tetraselmis*, *Isochrysis* but also *Thalasiossira weisflogii*. If no comparisons were performed with cultures fed by a conventionally treated seawater, the cultures could be monitored over the long term as shown in Figure 4.

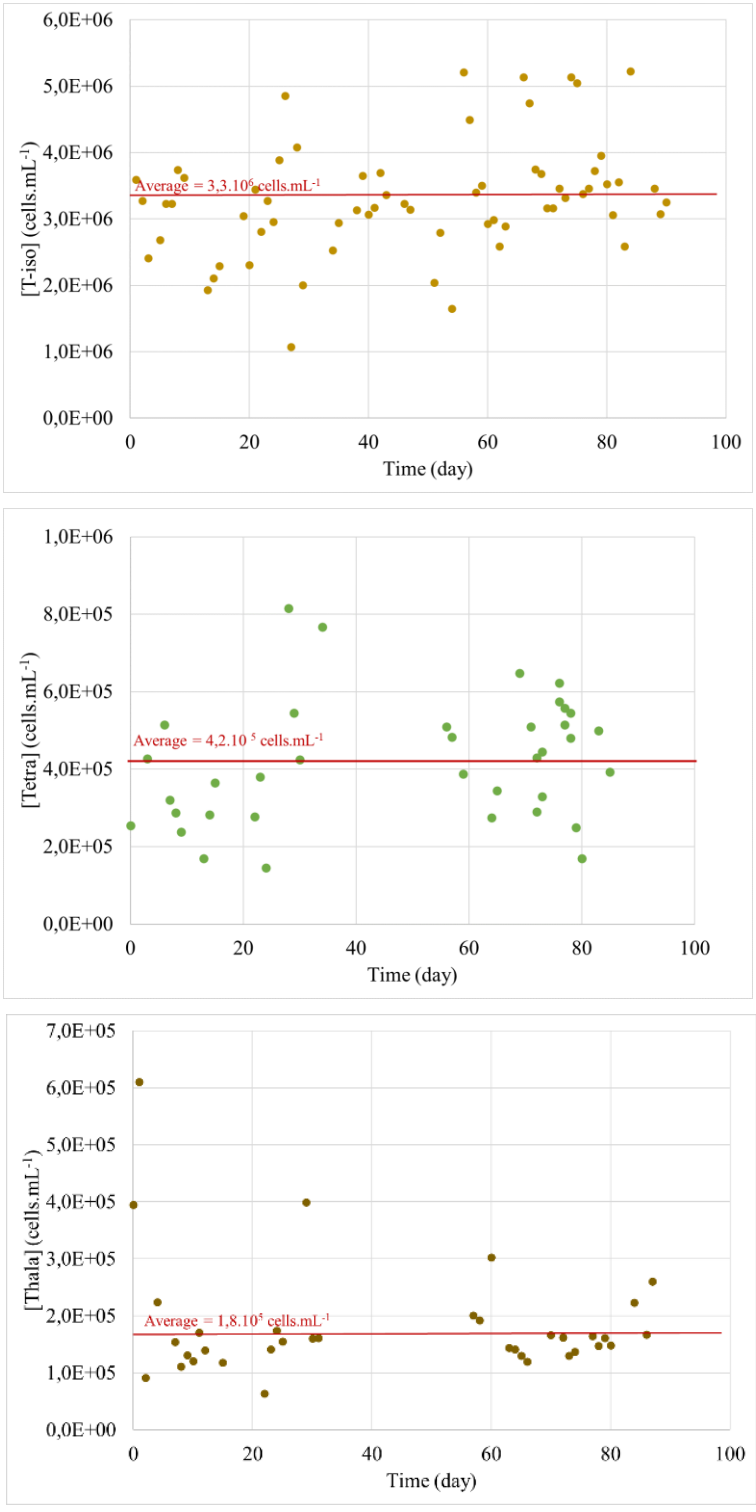


Figure 4. Monitoring of concentrations in Isochrysis, Tetraselmis and Thalassosiera weisfogii over 4 months

These measurements confirmed two results going in the direction of the microalgae cultures biosecurity: (i) The first, expected, is the stability of the culture on all the study period with a duration up to 3 weeks for *Isochrysis*, 2 weeks for *Tetraselmis* and 5 weeks for *Thalassosiera*. Otherwise, no other microalgae or predators were observed during the conservation of the strains in small volumes of 250 mL on the same period. (ii) The second is an increase of the cell concentration which agrees, or even stronger, with the preliminary comparison tests. Using Ultrafiltered seawater, the maximum concentrations reached and the growth kinetics can be estimated for *Isochrysis*, *Tetraselmis* and *Thalassosiera* respectively: (Iso – C_{max} = 8767000 cell.mL^{-1}; 700000 cell.mL^{-1}d^{-1}) (Tétra – C_{max} = 1980000 cell.mL^{-1}; 178000 cell.mL^{-1}d^{-1}) (Thala – C_{max} = 675830 cell.mL^{-1}; 46000 cell.mL^{-1}d^{-1})

4. Conclusion

In conclusion, ultrafiltration has shown its effectiveness for the protection of farms (Cordier *et al.*, 2019), it has appeared relevant to extend it to other applications within the shellfish farm. Thus, cultures in a volume of 300 L of 2 species of microalgae *Tetraselmis* and *T-isochrysis*, were monitored for the first time in UF seawater and in filtered-disinfected seawater as it's generally carried out within shellfish structures (prefiltration, 3 filtrations and 2 UV). The most important result is the securing of microalgea cultures with the absence of parasites in all cultures supplied with ultrafiltered water, unlike analyzes of the various control cultures. In the case of *T-isochrysis*, a higher microalgae concentration in ultrafiltered water, up to 30%, thus bringing a benefit on the algal density, is observed on 3 out of 4 tests. Moreover UF making easier the recovering of disinfected seawater (in agreement with the reduction of the treatment units), a transfer of technology and a modification of the cultures processes were decided on the study site (Experimental Marine Molluscs Platform Ifremer from Bouin), using ultrafiltered water since early 2019. Feedback, to date, after several months of culture with these two microalgae as well as a third, *Thalasossiera*, confirm the observations previously obtained since the cultures are stable and free from parasites.

5. Acknowledgements

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6. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

7. Ethical Approval

The manuscript does not contain experiments on animals and humans; hence ethical permission not required.

8. References

[1] Guedes Catarina, A, & Xavier, F. (2012). Nutritional Value and Uses of Microalgae in Aquaculture. Aquaculture. doi:10.5772/30576.

[2] Helm, M. M. (2004). Hatchery culture of bivalves: a practical manual (No. 639.2 F3 v. 471). FAO.

[3] Wallace, R. K., Waters, P., & Rikard, F. S. (2008). Oyster hatchery techniques. Southern Regional Aquaculture Center.

[4] Brown, M.R., Jeffrey, S.W., Volkman, J.K., Dunstan, G.A., (1997). Nutritional properties of microalgae for mariculture. Aquaculture, Fish Nutrition et Feeding Proceedings of the Sixth International Symposium on Feeding et Nutrition in Fish 151(1-4), 315–331. doi:10.1016/s0044-8486(96)01501-3.

[5] Wikfors, G. H., Patterson, G. W., Ghosh, P., Lewin, R. A., Smith, B. C., & Alix, J. H. (1996). Growth of post-set oysters, Crassostrea virginica, on high-lipid strains of algal flagellates Tetraselmis spp. Aquaculture, 143(3-4), 411–419. doi:10.1016/0044-8486(96)01265-3.

[6] Ifremer, (2013), Microalgues. Aquaculture. Available online: https://aquaculture.ifremer.fr/les-Filieres/Filiere-Algues/La-decouverte-des-algues/Microalgues (accessed on 7 September 2019).

[7] Mok, J. S., Ryu, A., Kwon, J. Y., Kim, B., & Park, K. (2019). Distribution of Vibrio species isolated from bivalves and bivalve culture environments along the Gyeongnam coast in Korea: Virulence and antimicrobial resistance of Vibrio parahaemolyticus isolates. Food Control, 106, 106697. doi:10.1016/j.foodcont.2019.06.023.

[8] Lumbessy, S. Y. (2019). Sem-sterile culture of Gracilaria salicornia seaweed (parental) on various media. Proceedings of the 2nd International Conference on Bioscience, Biotechnology, and Biometrics 2019. doi:10.1063/1.5141331.

[9] Wardani, A. K., Hakim, A. N., Khoiruddin, & Wenten, I. G. (2017). Combined ultrafiltration-electrodeionization technique for production of high purity water. Water Science and Technology, 75(12), 2891–2899. doi:10.2166/wst.2017.173.

[10] Greenlee, L. F., Lawler, D. F., Freeman, B. D., Marrot, B., & Moulin, P. (2009). Reverse osmosis desalination: water sources, technology, and today's challenges. Water research, 43(9), 2317-2348. doi: 10.1016/j.watres.2009.03.010.

[11] Ferguson, R. L., Buckley, E. N., & Palumbo, A. V. (1984). Response of marine bacterioplankton to differential filtration and confinement. Applied and Environmental Microbiology, 47(1), 49–55. doi:10.1128/aem.47.1.49-55.1984.

[12] Huq, A., Xu, B., Chowdhury, M. A., Islam, M. S., Montilla, R., & Colwell, R. R. (1996). A simple filtration method to remove plankton-associated Vibrio cholerae in raw water supplies in developing countries. Applied and Environmental Microbiology, 62(7), 2508–2512. doi:10.1128/aem.62.7.2508-2512.1996.

[13] Wenten, I. G., Steven, S., Dwiputra, A., Khoiruddin, & Hakim, A. N. (2017). From lab to full-scale ultrafiltration in microalgae harvesting. Journal of Physics: Conference Series, 877, 012002. doi:10.1088/1742-6596/877/1/012002.

[14] Cordier, C., Charpin, L., Stavrakakis, C., Papin, M., Guyomard, K., Sauvade, P., … Moulin, P. (2019). Ultrafiltration: A solution to recycle the breeding waters in shellfish production. Aquaculture, 504, 30–38. doi:10.1016/j.aquaculture.2019.01.045.

[15] Cordier, C., Stavrakakis, C., Dupuy, B., Papin, M., Sauvade, P., Coelho, F., & Moulin, P. (2019). Ultrafiltration for environment safety in shellfish production: Removal of oyster gametes in hatchery effluents. Aquacultural Engineering, 84, 80–90. doi:10.1016/j.aquaeng.2018.12.008.

[16] Cordier, C., Stavrakakis, C., Sauvade, P., Coelho, F., & Moulin, P. (2018). Air Backwash Efficiency on Organic Fouling of UF Membranes Applied to Shellfish Hatchery Effluents. Membranes, 8(3), 48. doi:10.3390/membranes8030048.

[17] Guilbaud, J., wyart, Y., Kaag, K., moulin, P. (2018). Comparison of Seawater and Freshwater Ultrafltration on Semi-Industrial Scale: Ballast Water Treatment Application. Journal of Membrane Science and Research, 4(3), 136-145. doi:10.22079/jmsr.2017.68010.1147.

[18] Guilbaud, J., Wyart, Y., & Moulin, P. (2018). Economic viability of treating ballast water of ships by ultrafiltration as a function of the process position. Journal of Marine Science and Technology, 24(4), 1197–1208. doi:10.1007/s00773-018-0618-3.