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Depuration of bivalve molluscs: a literature review

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Abstract

As they have an effective filter-feeding mechanism, bivalve molluscs can be used in the assessment of contamination of marine environments to measure parameters related to microbiological contaminants. Given this public health problem, a depuration system is necessary. This study aimed to conduct a literature review on depuration, the systems used, the water disinfection processes combined with a sanitizing agent, such as chlorine, ultraviolet light (UV) or ozone. Therefore, depuration can be an efficient way to reduce pathogenic bacteria in molluscs, but more studies should be conducted to confirm the efficiency of this process under non-experimental conditions and on an industrial scale.

Keywords: chlorine; contamination; disinfection; UV light; ozone.

Practical Application: The water depuration processes combined with a sanitizing agent such as chlorine, ultraviolet (UV) light or ozone are an efficient way to reduce pathogenic bacteria in molluscs.

1 Introduction

Bivalve molluscs are filter-feeding aquatic invertebrates characterized by the presence of shells formed by two valves. Mussels, oysters, cockles, and scallops are examples of bivalves (Brasil, 2012).

These filtering organisms feed on sediments from the water column, capturing small phytoplankton and zooplankton species. In this process, they absorb biological and chemical pollutants, toxins, heavy metals and microorganisms present in the water (Toro et al., 2003; Cortes et al., 2009; Dame, 2012).

Among the microorganisms, pathogens such as *Escherichia coli*, *Salmonella* spp., *Staphylococcus* spp. and *Vibrio* spp., deserve special attention, as they compromise food safety and security. Thus, bivalve molluscs may be associated with foodborne disease outbreaks. One of the tools for the control of the causative agents of diseases is the use of preventive measures in the production chain and the education of the individuals involved in the food production process.

In the production and marketing of bivalve molluscs, Brazilian legislation includes safety regulations regarding the presence of microorganisms in fish products. The National Health Surveillance Agency (ANVISA), through RDC No. 331, of December 23, 2019, which provides for the microbiological standards of foods and their application, is complemented by Normative Instruction No. 60, of December 23, 2019, which establishes the lists of microbiological standards for foods (Brasil, 2019a; Brasil, 2019b).

Moreover, Interministerial Normative Instruction MPA/MAPA No. 07, of May 8, 2012, which institutes the National Programme for Hygiene and Sanitary Control of Bivalve Molluscs (PNCMB) aims to establish the minimum requirements necessary to guarantee the safety and quality of bivalve molluscs for human consumption (Brasil, 2012).

However, relevant international legislation is extremely strict, given the high number of cases of diseases associated with the consumption of contaminated seafood (Richards, 2003; Younger et al., 2003). Most countries that produce foods of marine origin have their own legislation based on regulations from large markets such as the United States and the European Union.

The European Directive 91/492/EEC, of July 15, 1991 (European Communities, 1991) sets out hygiene rules for the production and placing on the European Common Market of live bivalve molluscs. The areas destined for cultivation are classified according to the microbiological quality of the meat of molluscs produced in these waters.

The main strategy of depuration is to control the risks of shellfish sold to final consumers and consumed live, such as oysters. Cockles or mussels that are purchased alive and eaten cooked by the consumers must also be submitted to the depuration process, as commercialization does not guarantee the elimination of the causative agents of diseases (Souza et al., 2021).

However, the United States counts on a National Shellfish Sanitation Program, with regulations based on interstate commercial agreements recognized by the FDA (National Shellfish Sanitation Program, 2003). This program aims to promote and value the production of shellfish in the country, standardizing regulations among the American states.

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According to Souza et al. (2021), the best way to produce safe mollusks is to grow them in unpolluted waters. However, many coastal areas close to cities are impacted by anthropogenic pollution, and hence bivalve mollusks are contaminated by these etiological agents. The present study aimed to perform a literature review on depuration in bivalve mollusks from cultivation areas that pose a risk to the consumers' health.

2 Depuration

Depuration is a technique used to reduce microbial contamination of filtering molluscs, to levels acceptable by legislation for human consumption, by keeping the animals in tanks with clean water (Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina S.A., 2013). Since depuration is a way to ensure product quality, protocols applicable to the local product are needed.

The recommended minimum water flow rate in the United States and New Zealand is 107 liters/minute per cubic meter of animals. In Japan, the minimum flow rate described is 12 liters/minute per 1,000 oysters (Souza et al., 2021).

Marine water of good quality must be available to ensure that the depuration process reduces the contamination of bivalve molluscs. However, seawater with adequate composition and highly transparent (with low turbidity) is required, especially in establishments that use ultraviolet light disinfection. However, when the seawater available in the region does not have adequate transparency and salinity, or when the purification establishment is far from the sea, artificial seawater can be used (Souza et al., 2021).

According to Rodrick & Schneider (2003), salinity, water temperature, dissolved oxygen content, turbidity and phytoplankton concentration can impact the process of elimination of pathogens. These factors must be controlled, as the filtration rate, physiological activity and behavioral responses of molluscs may vary depending on the depuration environment. Regarding microbiological pathogens, potentially relevant hazards are shown in Table 1.

The process of depuration depends on the level of risks related to fecal pollution that affects the areas of cultivation or extraction of bivalve molluscs. This risk is determined through the National Programme for Hygiene and Sanitary Control of Bivalve Molluscs (PNCMB), which aims to establish the minimum requirements necessary to guarantee the safety and quality of bivalve molluscs for human consumption (Brasil, 2012).

As for the depuration time, it must be long enough for the molluscs to release pathogens from the intestinal tract. Therefore, based on the results of studies on the elimination of bacteria that are indicators of pollution (such as coliforms) and other pathogenic microorganisms (marine viruses and bacteria), the

Table 1. Pathogen Matrix.

HAZARD RANKING	PATHOGEN	EVIDENCE OF BIVALVE-ASSOCIATED ILLNESS	FREQUENCY OF BIVALVE- ASSOCIATED ILLNESS	SEVERITY OF ILLNESS	LIKELY SOURCE OF CONTAMINATION TO THE AQUATIC ENVIRONMENTS
	Norovirus (also for Sapovirus and Aichivirus)	Yes	Common	It is usually not severe	Human feces
	Hepatits A Virus	Yes	Moderately common in endemic areas	Moderately severe	Human feces
Primary hazards	*Salmonella typhi* e *Salmonella paratyphi*	Yes	Moderately common in endemic areas	Severe	Human feces
	Other *Salmonella* serotypes	Yes	Moderately common	It is usually not severe	Human or animal feces
	Vibrio parahaemolyticus	Yes	Moderately common	It is usually not severe	Autochthonous
	Vibrio vulnificus	Yes	Rare	Severe	Autochthonous
	Toxigenic *Vibrio cholerae*	Yes	Moderately common in endemic areas	Severe when there is no adequate medical support or support	Human feces, sometimes Autochtonous
	Other *Vibrio* species	Yes	Moderately common	It is usually not severe	Autochthonous
Secondary hazards	*Campylobacter* spp.	Yes	Rare	It is usually not severe	Human or animal feces
	Listeria monocytogenes	Yes	Rare	It is usually not severe	Animal feces
	Giardia intestinalis	Yes	Rare	It is usually not severe	Human or animal feces
	Hepatitis E Virus	No	Not applicable	Moderately severe	Animal feces (pig, wild boar, deer and rats)
	Enterocolytic *Yersinia*	No	Not applicable	It is usually not severe	Animal feces
Potential hazards	*Microsporidia*	No	Not applicable	It is usually not severe	Animal feces
	Cryptosporidium parvum	No	Not applicable	It is usually not severe	Human or animal feces
	Toxoplasma gondii	No	Not applicable	It is usually not severe	Animal feces (mainly cats)

1 Frequency expected when an adequate sanitation program is not available. Frequency will vary significantly by continent, country and even region. It can also change over time.
2 Severity expected in immunocompetent individuals without another underlying disease. Not applicable to *V. vulnificus*, which usually only causes symptomatic disease in immunocompromised people or people with underlying diseases.
3 The term animal is used to comprise mammals and birds. However, some enteric pathogens (eg, some *Salmonella enterica* serovars) can be carried by cold-blooded animals such as reptiles.
4 The frequency of norovirus and hepatitis A infection will be reduced only partially by a program based on fecal indicator bacteria.
5 These pathogens can produce severe and/or chronic diseases in some patients.
6 It can be severe in immunocompromised patients and can have serious consequences for the fetus if a pregnant woman becomes infected.
7 *Toxoplasma gondii* can cause serious health problems for the fetus if the infection occurs in a pregnant woman.

Source: Adapted from FAO and WHO (Food and Agriculture Organization, 2018a).

different countries can determine the minimum periods or time required for the depuration process (Souza et al., 2021).

According to the FAO (Food and Agriculture Organization, 2018b), the depuration process that is currently commercially practiced removes many fecal bacterial contaminants and is less effective in removing viral contaminants such as noroviruses and hepatitis A. Yet, this process cannot remove other contaminants such as naturally occurring marine vibrios (e.g. *Vibrio vulnificus* and *Vibrio parahaemolyticus*), marine biotoxins (such as the toxins that cause paralytic shellfish poisoning - PSP, diarrheal shellfish poisoning - DSP and amnesic shellfish poisoning - ASP, or heavy metals or organic chemicals).

Consequently, depuration is intended to reduce only the risks related to fecal pollution. It is not a viable means to reduce contamination by toxins produced by microscopic algae during algal bloom events (red tides) and other contaminants such as heavy metals (Souza et al., 2021).

3 What are the depuration systems and who is legally responsible to perform this procedure?

There are different types of depuration systems, as follows: the open system - with constant flow of water; the closed system - a closed recirculated seawater system or the "Batch-process" system where water is replaced at regular intervals. The closed recirculation system is currently the most used because it uses less water (Corrêa et al., 2007).

According to the Brazilian legislation, a depuration plant is the establishment intended for the reception, purification, packaging, labeling, storage and shipping of bivalve molluscs (Brasil, 2020). The seafood processing sector is responsible for the depuration procedure of bivalve molluscs before they are taken to restaurants and fish markets. Consequently, mariculturers are not responsible for the referred procedure (Brasil, 2012).

4 Processes for water disinfection

The first application of UV light for the disinfection of water occurred in 1910, in Marseille, France. This was possible thanks to the development of mercury lamps and quartz tubes. However, due to the high cost of the procedure, and with the advent of chlorine, the procedure was discontinued. Studies have shown that treatment with chemical agents such as chlorine can generate toxic by-products, and thus UV light treatment has gained commercial interest on a larger scale (Hijnen et al., 2006; Hassen et al., 2000).

According to Upadhyaya et al. (2004), UV radiation acts on the nucleic acids of microorganisms, causing progressive damage to bacterial cells. These cells absorb a high dose of radiation, which leads to a rupture of the cell membrane, causing biological death. The mechanism of action is the one that generates double-stranded DNA breaks and thymine dimer formation.

The UV light equipment consists of overlapping tubes: an external tube (usually made of plastic) and a transparent internal tube made from quartz, where a UV lamp is placed. Exposure to radiation depends on the power of the lamp and the time taken for water to travel through the space between the external and internal tubes (Souza et al., 2021).

Ozone (O_3) is an unstable allotropic form of oxygen (O_2), that is, t is made up of the same atoms, but they are combined differently and the difference is in the number of atoms.

Therefore, with an extra atom, ozone is considered enriched oxygen. This gas is colorless and has a pungent odor, molecular weight of 48, liquefies at -112 °C and has a freezing point of -251.4 °C. It decomposes rapidly, in a violent or explosive reaction, at temperatures above 100 °C, or, in the presence of some catalysts, at room temperature (Gonçalves & Paiva, 2004).

In Europe, ozone has been used to disinfect drinking water. In Brazil, despite several reports of commercial applications, such as sterilization of swimming pools and water bottling, its use is almost nonexistent, with chlorine being the most used sanitizer in industries, although during its handling, toxic compounds can be formed that produce unwanted contaminants such as the formation of trihalomethanes (Gonçalves & Gagnon, 2011).

Ozone gas can be produced by an ozone generator or purchased in cylinders. When diffusers are added to seawater, bacteria and viruses are inactivated. The gas is mostly used in depuration centers for large quantities of bivalve molluscs, and very strict safety protocols are adopted (Souza et al., 2021).

As an antimicrobial agent, ozone acts directly on the cell wall, causing its rupture and destruction, at shorter time contact. (Snatural Tecnologias Ambientais Ltda, 2013).

Studies have shown the bactericidal effect of ozone on a wide variety of microorganisms, including Gram-negative and Gram-positive bacteria. Gram-negative bacteria are more sensitive to ozone compared to Gram-positive bacteria, as they have less peptide glycan in their cell wall (Silva et al., 2011).

The chemical water disinfection method for the depuration of molluscs uses chlorination, due to its disinfectant capacity and easy handling. However, chlorine impairs molluscs' filtering ability, impactis the quality of the end product, and may modify the appearance and taste of seafood. Thus, alternative methods of water purification should be explored, to minimize sensory alterations of molluscs at the end of the process (Suplicy, 1998).

Chlorine acts by diffusion, passing through cell membranes. Once it enters the cell, it disrupts its vital elements, such as enzymes, proteins, DNA and RNA (Snatural Tecnologias Ambientais Ltda, 2013).

5 Comparison of methods

Water disinfection methods have advantages and disadvantages that deserve attention when a depuration system is considered (Table 2). The main relevant aspects are implementation cost, operational cost, efficiency, residual effects and necessary contact time.

Table 2. Comparison between water disinfection methods.

Operation/Condition	Chlorine based products	UV light	Ozone
Operational costs	Low	The lowest	High
Investment costs	Medium	Low	High
Installation	Complex	Simple	Complex
Ease of maintenance	Moderate	Easy	Difficult
Maintenance cost	Medium	Low	High
Need for transparent water	Low	High	Medium
Performance	Possible increase	Excellent	Unreliable
Risks to employees	High	Medium (eyes and skin)	Medium (oxidant)
Toxic chemicals	Yes	No	Yes
Virucidal effect	Bad	Good	Good
Residual effect	Yes	No	Yes
Effect on water	Toxic byproducts	None	Toxic byproducts
Operational problems	Medium	Low	High
Contact time	30-60 minutes	1-5 seconds	10-20 minutes
Effect on molluscs	Irritating	None	Oxidant

Source: Adapted from Lee et al. (2008).

6 Final and future considerations

Given the fast growth of malacoculture, it is necessary to ensure that the areas of cultivation and production of bivalve molluscs have a microbiological quality consistent with the current Brazilian legislation.

Thus, consumer safety will depend on the quality of the product, which is related to the physical, chemical and microbiological conditions of the original environment.

To ensure that the export of bivalve molluscs is competitive in the international market, all guidelines must also comply with the applicable legislation of trading partners. The main markets for bivalve molluscs are the United States and the European Union, and depuration of molluscs in these countries guarantees a final product with high commercial value and in good sanitary conditions.

Nonetheless, the depuration process has the main purpose of controlling the risks to bivalve molluscs commercialized and sold to consumers, eliminating pathogenic etiological agents that cause diseases.

Therefore, the three main water disinfection methods used in depuration are ozone, ultraviolet light and chlorine-based compounds. Shellfish business operators are responsible for the depuration procedure of bivalve molluscs before they are taken to restaurants, fish markets and the final consumers.

References

Brasil. Ministério da Agricultura, Pecuária e Abastecimento. (2020). Altera o decreto n° 9.013, de 29 de março de 2017, que regulamenta a Lei nº 1.283, de 18 de dezembro de 1950, e a Lei nº 7.889, de 23 de novembro de 1989, que dispõem sobre a inspeção industrial e sanitária de produtos de origem animal (Decreto-lei n° 10.468, de 18 de agosto de 2020). *Diário Oficial [da] República Federativa do Brasil*.

Brasil. Ministério da Pesca e Aquicultura. (2012). Institui o Programa Nacional de Controle Higiênico-Sanitário de Moluscos Bivalves (PNCMB), estabelece os procedimentos para a sua execução e dá outras providências (Instrução normativa interministerial MPA/MAPA nº 7, de 8 de maio de 2012). *Diário Oficial [da] República Federativa do Brasil*.

Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária – ANVISA. (2019a). Dispõe sobre os padrões microbiológicos de alimentos e sua aplicação (RDC nº 331, de 23 de dezembro de 2019). *Diário Oficial [da] República Federativa do Brasil*, n° 249.

Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária – ANVISA. (2019b). Estabelece as listas de padrões microbiológicos para alimentos (Instrução Normativa n° 60, de 23 de Dezembro de 2019). *Diário Oficial [da] República Federativa do Brasil*.

Corrêa, A. A., Albarnaz, J. D., Moresco, V., Poli, C. R., Teixeira, A. L., Simões, C. M. O., & Barardi, C. R. M. (2007). Depuration dynamics of oysters (Crassostrea gigas) artificially contaminated by *Salmonella enterica* serovar *Typhimurium*. *Marine Environmental Research*, 63(5), 479-489. http://dx.doi.org/10.1016/j.marenvres.2006.12.002. PMid:17280712.

Cortes, M. B. V., Wasserman, J. C., & Avelar, J. C. L. (2009). Gestão da qualidade de moluscos bivalves de cultivos da Baía Da Ilha Grande (Paraty, Angra Dos Reis e Mangaratiba). In *Anais do V Congresso Nacional de Excelência em Gestão* (pp. 1-12). Niterói, RJ.

Dame, R. F. (2012). *Ecology of Marine Bivalves: an ecosystem approach* (2nd ed.). Routledge: Taylor & Francis Group.

Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina S.A. – EPAGRI. (2013). *Informativo EPAGRI (Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina S.A.): Síntese Informativa da Maricultura*. Florianopolis: EPAGRI.

European Communities. (1991). Council Directive of 15th of July 1991 laying down the health conditions for the production and placing on the market of live bivalve mollusks (91/492/EEC). *Official Journal of the European Union,* L268, 1-14.

Food and Agriculture Organization – FAO, & World Health Organization – WHO. (2018a). *Technical guidance for the development of the growing area aspects of Bivalve Mollusc Sanitation Programmes* (Food Safety and Quality Series, n° 5, 292 p.). Rome: FAO.

Food and Agriculture Organization – FAO. (2018b). *The state of world fisheries and aquaculture 2018 - meeting the sustainable development goals* (224 p.). Rome: FAO.

Gonçalves, A. A., & Paiva, F. G. (2004). El ozono como agente antiséptico em La indústria pesquera. *Infopesca Internacional*, 31(1), 32-37.

Gonçalves, A. G., & Gagnon, G. A. (2011). Ozone application in recirculating aquaculture system: an overview. *Ozone: Materials Science and Engineering*, 33(5), 345-367.

Hassen, A., Mahrouk, M., Ouzari, H., Cherif, M., Boudabous, A., & Damelincourt, J. J. (2000). UV disinfection of treated wastewater in a large scale pilot plant and inactivation of selected bacteria in a laboratory UV device. *Bioresource Technology*, 74(2), 141-150. http://dx.doi.org/10.1016/S0960-8524(99)00179-0.

Hijnen, W., Beerendonk, M., & Medema, G. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan oocysts in water: a review. *Water Research*, 40(1), 3-22. http://dx.doi.org/10.1016/j.watres.2005.10.030. PMid:16386286.

Lee, R., Lovatelli, A., & Ababouch, L. (2008). *Bivalve depuration: fundamental and practice aspects* (FAO Fisheries Technical Paper). Rome: FAO. Retrieved from http://www.fao.org/documents/card/en/c/91ea7200-8fdb-55ce-857b-a5816e157609/

National Shellfish Sanitation Program – NSSP. (2003). *Guide for the control of molluscan shellfish*. Silver Spring, MD: FDA.

Richards, G. P. (2003). The Evolution of molluscan shellfish safety. In A. Villalba, B. Reguera, J. L. Lopez-Romalde, &, R. Beiras (Eds.), *Molluscan shellfish safety* (pp. 221-322). Santiago de Compostela: Xunta de Galícia and Intergovernamental Oceanographic Commision of UNESCO.

Rodrick, G. E., & Schneider, K. R. Molluscan Shellfish Depuration. In A. Villaboa, B. Reguera, J. Romalde, & R. Reis (Eds.), (2003, June 4-8). *Proceedings of the 4th International Conference on Molluscan Shellfish Safety*. Santiago de Compostela: Consellería de Pesca y Asuntos Maritimos de Xunta de Galicia and Intergovernamental Oceanographic Commision of UNESCO.

Silva, S., Luvielmo, M., Geyer, M., & Pra, I.. (2011). Potencialidades do uso do ozônio no processamento de alimentos. *Semina: Ciências Agrárias*, 32(2), 659-682. http://dx.doi.org/10.5433/1679-0359.2011v32n2p659.

Snatural Tecnologias Ambientais Ltda – SNATURAL. (2013). *Ozônio*. Retrieved from https://www.snatural.com.br/ozonio-tratamento-agua-desinfeccao/

Souza, R. V., Suplicy, F. M., & Novaes, A. L. T. (2021). *Depuração de moluscos bivalves* (Boletim Didático, 160, 70 p.). Florianópolis, SC: EPAGRI.

Suplicy, F. M. (1998). *Ensaios sobre a depuração do mexilhão Perna perna (L., 1758)* (Dissertação de mestrado). Universidade Federal de Santa Catarina, Florianópolis.

Toro, B., Navarro, J. M., & Palma-Fleming, H. (2003). Relationship between bioenergetics responses and organic pollutants in the giant mussel, *Choromytilus chorus* (Mollusca: Mytilidae). *Aquatic Toxicology (Amsterdam, Netherlands)*, 63(3), 257-269. http://dx.doi.org/10.1016/S0166-445X(02)00181-9. PMid:12711415.

Upadhyaya, G. S., Curry, R. D., Nichols, L., Clevenger, T. E., & Mcdonald, K. F. (2004). The design and comparison of continuous and pulsed ultraviolet reactors for microbial inactivation in water. *IEEE Transactions on Plasma Science*, 32(5), 2032-2037. http://dx.doi.org/10.1109/TPS.2004.835951.

Younger, A. D., Lee, R. J., & Lees, D. N. (2003). Microbiological monitoring of bivalve shellfish harvesting areas in England and Wales – rational and approach. In A. Villalba, B. Reguera, J. L. Lopez-Romalde, & R. Beiras (Eds.), *Molluscan Shellfish Safety*. Santiago de Compostela: Conselleria de Pesca da Xunta de Galícia and Intergovernamental Oceanographic Commision of UNESCO.