Halophilic Bacteria on Natural Hog Casings Industry Wastewater Treatment

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Abstract. This literature review article presents the characteristics of a natural hog casings industry wastewater, mainly its salinity. With a chloride concentration of 6.9% w/v, the wastewater from this industry presents challenges for conventional biological processes due to its high salinity. The study explores halophilic microorganisms, their osmoregulation strategies, mainly the salt-in strategy, which allow them to survive in high-salt environments by matching external concentrations. Four species of anaerobic halophilic bacteria that use the salt-in strategy, *Haloanaerobium praevalens, Haloanaerobium acetobutylicum, Haloanaerobacter salinarius* and *Orenia marismortui*, were identified as promising for treating this specific wastewater. The conclusion suggests that these bacteria can consume organic matter and in addition Na+ and Cl^- from the effluent, reducing salinity.

Keywords. Biotreatment, Saline wastewater, Salt-in strategy, *Haloanaerobic*.

1. Introduction

Salt, also known as table salt or sodium chloride (NaCl), has been used as a seasoning and preservative for meats, fish, vegetables, and other foods since ancient times (1). In industrial food processing, salting is also used to inhibit the growth of microorganisms by reducing water activity (a_w), defined as the amount of free water available for microbial growth (2). However, some species have adapted to this environment; these are the halophiles, salt-loving microorganisms.

Halophilic microorganisms can include bacteria, archaea and eukarya, and are categorized according to the salt concentration required for optimal growth. Halotolerant organisms can grow in NaCl concentrations ranging from 1.17% to 2.93%, but rather live in the absence of it. Moderate halophiles thrive in concentrations between 2.93% and 14.63% NaCl, while extreme halophiles require concentrations between 14.63% and 30.4% NaCl (3–5).

In the natural hog casings industry, salt and brine are used to preserve the goods from microbial growth. After processing has been completed, the casings are salted and then packed either in dry salt or with fully saturated brine. In the industry, degrees Baumé (°Bé) is used to measure the concentration of NaCl in the brine and it must be at least 22 °Bé to ensure a a_w between 0.80 and 0.75. This a_w for 30 days ensure that some microorganism as *Bacillus cereus*, *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, *Listeria monocytogenes* and others can not survive to this hostile environment (6).

Due to the amount of salt used to ensure the quality of the casings, a large amount of brine and salt ends up in the wastewater treatment, as they are applied and naturally lost in production processes. Considering analyses performed by a Brazilian natural hog casings industry over a period of 5 months, an average of 43 m^3 of effluent is generated per day, with an average of 69 g/L of free chloride (Cl^-). The wastewater treatment of this industry is unable to remove all the Cl^- with the current biological treatment.

This literature review aims to analyze and discuss the possible wastewater treatment generated by a specific Brazilian natural hog casings industry with halophilic bacteria.

2. Natural Hog Casings Wastewater's Characteristics

To characterize the gross effluent, four analyses were performed over 5 months in 2024 and the results are shown in Table 1. The most important parameters that characterize this effluent are Cl^- and COD.

2.1 Cl^-

Cl^- levels are directly related to salinity, as NaCl dissolves in aqueous media, resulting in free Cl^-. Therefore, the concentration of Cl^- is considered the same as that of NaCl. Seawater contains 3.5% w/v of dissolved salts, concentration that remains relatively

Tab. 1 - Characteristics of the effluent

Sample	Cl^- (mg/L)	COD (mg/L)	BOD (mg/L)	Total phosphorus (mg/L)	Ammoniacal nitrogen (mg/L)	pH
1st pickling	87,840	2,380	530	48.2	59.8	6.4
2nd pickling	94,010	2,640	40.8	43.7	1.5	7.0
3rd pickling	50,886	1,775	536	37.5	10.7	6.9
4th pickling	45,130	2,682	1,260	32.4	8.2	6.5
Average	69,467	2,369	592	40.4	20	6.7

constant worldwide, but is insufficient to sustain the survival of extreme halophiles (7). The effluent analyzed has approximately 6.9% (w/v) Cl^- in its composition, indicating that moderate halophilic microoganisms would adapt to the environment.

2.2 COD & BOD

Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) are used to measure the amount of organic matter present in the effluent. BOD reflects the biochemical oxidation of organic matter, performed entirely by microorganisms. COD, however, corresponds to the chemical oxidation of organic matter. BOD is included within COD, since biological demand originates from chemical availability (8).

COD in wastewater from swine slaughterhouse in Brazil, according to João et al. (9), is 4,380 ± 326 mg/L and, according to Oliveira et al. (10), it is 7,176 ± 4,631 mg/L. While the COD of a Brazilian domestic effluent according to Silva (11) is 553.28 mg/L, according to Bezerra et al. (12) it is 758.25 mg/L and according to Kikuti (13) it is 976.5 mg/L. Comparing the CODs of domestic effluents and those of swine slaughterhouses, it can be observed that, despite being an industry, the effluent of interest in this article has a COD closer to that of a domestic effluent than that of a slaughterhouse.

The COD/BOD ratio is used to evaluate the biotreatability of an effluent. This ratio for domestic sewage varies between 1.7 and 2.4, but for industrial effluents can vary widely. The lower the COD/BOD ratio, the higher the biodegradable fraction and the greater the indication for biological treatment (8). Considering the average COD and BOD of the effluent analyzed, a ratio of 4.002 is obtained, result that does not indicate biological treatment for this effluent. However, when the samples are analyzed individually in terms of the COD/BOD, there is a wide variation in the results. In the last sample, the ratio was 2.1, which indicates the possibility of biotreatment.

3. Biotreatment of Natural Hog Casings Wastewater

3.1 Halophilic Microorganisms Survival Strategies

High salt concentrations cause osmotic stress in the microorganisms that live in that environment. In order to survive in the saline environment, there are two distinct strategies that allow these organisms to survive (14).

- Compatible-solute or salt-out strategy: cells maintain low intracellular salt levels and balance external osmotic pressure with organic solutes, avoiding the need for intracellular adaptation. This strategy is more common among moderate halophiles, as well as marine microbes (14,15).
- Salt-in strategy: cells maintain high intracellular levels of salt, matching external concentrations and requiring adaptation to high salt content. It is commonly used by hyper-halophiles and considered the typical archaeal strategy of osmoadaptation (14,15).

3.2 Salt-in Strategy Microorganisms

Archaea that use this strategy maintain a low concentration of Na^+ in their cytoplasm, with 100 times more K^+ in their cells than in the surrounding environment. However, anaerobic halophilic bacteria, despite generaly maintaining K^+ as the predominant ion in their cytoplasm, also have a high concentration of Na+, and when they reach a stationary level, their cells eventually replace K^+ with Na^+ (15).

Anaerobic fermentative halophilic bacteria from the *Haloanaerobic* family have higher intracellular Cl^- concentrations than aerobic halophilic and halotolerant eubacteria, which tipically contain much lower Cl^- concentrations than the external environment (16–18).

Considering that biological effluent treatment takes place under normal temperature conditions and that the characteristics of the raw effluent are already known, four species of haloanaerobic bacteria were chosen that have growth and development characteristics closest to those of the effluent. Table 2 shows the characteristics of the four species of anaerobic halophiles. The most important criteria were the NaCl range, the temperature range and then the pH range.

Tab. 2 - Characteristics of anaerobic halophiles

Characteristic	*Haloanaerobium praevalens*	*Haloanaerobium acetobutylicum*	*Haloanaerobacter salinarius*	*Orenia marismortui*
Gram stain	Positive	Positive	Negative	Negative
NaCl range (%)	2-30	5-22	5-30	3-18
Ideal NaCl (%)	13	10	14-15	3-12
Temp. range (°C)	5-60	15-45	10-50	25-50
Ideal temp. (°C)	37	34	45	36-45
pH range	6.0-9.0	5.4-8.0	6-8	NR[a]
Ideal pH	7.0-7.4	6.3-7.4	7.4-7.8	NR[a]
References	(15,19)	(20–22)	(23)	(22)

NR – not reported

Haloanaerobium praevalens is a fermentative bacterium and although it is a Gram-positive, it has a Gram-negative cellular architecture (19). According to Oren, Heldal and Norland (16), in cells in exponential phase with 1.74 ± 0.32 pg/cell dry matter, the intracellular concentration of K+, Na+ and Cl^- were 1.136 ± 0.216 M, 0.438 ± 0.131 M and 1.263 ± 0.256 M, respectively. In stationary phase cells with 2.05 ± 0.69 pg/cell dry matter, the concentrations of K^+, Na^+ and Cl^- were 0.267 ± 0.220 M, 0.953 ± 0.420 M and 1.010 ± 0.362, respectively.

Haloanaerobium acetobutylicum, formerly known as *Halobacteroides acetoethylicus* (24) is a Gram-positive fermentative bacteria and, according to Rengpipat (17), has intracellular salt concentrations very similar to those of its environment. At an ionic NaCl concentration of 1.7 M, the intracellular values of K^+, Na^+ and Cl^- were 0.240 M, 0.92 M and 1.2 M, respectively, while in the surrounding medium the concentrations of the same elements were 0.032 M, 1.16 M and 1.4 M, respectively (17).

Haloanaerobacter salinarius and *Orenia marismortui* are Gram-negative fermentative bacteria (22,23) that have ideal or near-ideal characteristics for the effluent, but their osmoregulation has not yet been studied.

4. Conclusion

This literature review shows that wastewater from a natural hog casing industry contains 6.9% Cl^-, directly correlated to the concentration of NaCl, which is twice the salinity of seawater. Analysing the COD/BOD ratio, the effluent oscillates between being biotreatable and not.

Halophilic microorganisms have two possible strategies for osmoregulation, known as compatible-solute or salt-out and salt-in strategy, in which intracellular pressure is balanced with that of the external environment.

Anaerobic fermentative halophilic bacteria from the *Haloanaerobic* family have higher intracellular Cl^- concentrations than aerobic halophilic and halotolerant eubacteria. *Haloanaerobium praevalens*, *Haloanaerobium acetobutylicum*, *Haloanaerobacter salinarius* and *Orenia marismortui*, all have growth and development characteristics appropriate to the conditions provided by the effluent.

In conclusion, experimental studies should be carried out considering haloanaerobacteria in order to obtain results on the consumption of organic matter, Na^+ and Cl^- from the effluent.

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