APPLICATION OF HALOPHILE MICROORGANIMS OF AQUATIC ENVIRONMENTS

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OBJECTIVES

The aim of this review is to state how halophilic microorganisms survive at high salt concentrations. It also intends to do a review of some of their applications regarding the fields of environment, industry and medicine.

INTRODUCTION

Halophiles are salt-loving organisms that can life in saline environments and can be classified on their requirement for salt.

	Nonhalophiles	Halotolerant	Halophiles	Extreme halophiles
Optimum salt concentration for growing	> 0.2 M NaCl	0.2 - 0.5 M NaCl	0.5 - 2.5 M NaCl	2.5 - 5.5 M NaCl

Table 1. Classification regard salt concentration requirement for optimum growth.

The phylogenetic diversity of these microorganisms is huge as we can find them in all three domain of life: Eukarya, Bacteria and Archaea. Their metabolic diversity is also high, there only few dissimilatory processes that haven't been yet found in high salt concentrations.

In order to survive at high salt concentration there are basically two strategies:

"High-salt in" strategy

- Accumulation of ions like K^+ and Cl^-
- Low energetic cost
- Use limited to some Archaea
- Requires resistant proteins

"Low-salt, high organic solutes in" strategy

- Accumulation of compatible solutes
- High energetic cost
- Used in all three domains

Apart from these two strategies, in all studied cases, halophiles excluded Na^+ from the cytoplasm.

ENVIRONMENTAL APPLICATIONS

DECONTAMINATION OF SALINE WASTEWATER

Saline wastewater is generate from industrial activities and it is rich in both organic matter and salt. These normally has been treated through physico-chemical treatments, however it has a high cost and some of them have secondary effect because they are potential carcinogens. The biological treatment avoids these secondary effects and the cost is lower.

To prove its effectivity an experiment with saline wastewater were performed. Two different cultures were used, an activate sludge, and a combination of an *Halobacter* supplemented activated-sludge culture. To analyze effectiveness the chemical oxygen demand (COD) removal is measured. The results, in *figure 2*, show that the *Halobacter* supplemented activated sludge culture had better COD removal rates and efficiencies at all salt concentrations, while the activated sludge culture lost its effectivity as salt concentration was increased.

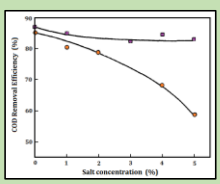
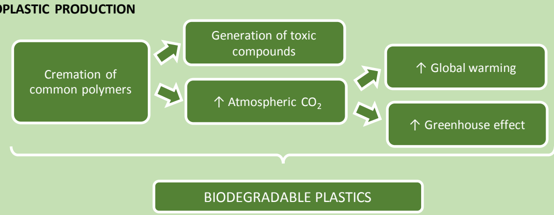


Figure 2. Variation of COD removal efficiency with salt concentration after treatment of saline wastewater.
Activated sludge
Activated sludge + *Halobacter*
Modified from [2].

In conclusion, addition of *Halobacter* to an activate sludge culture used for biological treatment of saline wastewater was proven to be advantageous, specially at salt concentration above 1%.

Unfortunately, most of the experiments about biological treatment od saline wastewater are laboratory-scale only, due to the complexity of the contaminated site.

BIOPLASTIC PRODUCTION



The production of biodegradable polymers such as polyhydroxyalkanoates (PHAs) which are produced naturally as a storage polymer, is a good solution as bioremediation, since it will help to decrease the harmful effects of the common polymers.

The production of PHA is well extended in the prokaryotic life, and it includes the halophile microorganisms. For this application was studied the genres like *Haloferax*, *Halobacterium*, *Haloarcula* and *Haloquadratum*. Among these, *Haloferax mediterranei* was the most promising archean for industrial PHA production.

H. mediterranei grows quickly and can accumulates in its cytoplasm large amounts of PHA using inexpensive materials as a source of carbon. The cultivation medium is rich at salt so there is little danger of contamination. Furthermore, like other halophiles, this microorganism is easily lysed in absence of salt releasing the PHA granules which makes the acquisition and purification of the polymer relatively simple.

MEDICAL APPLICATION

ARTIFICAL RETINA

Blindness for retinal photoreceptor deterioration like Age-related Macular Degeneration and Retinitis Pigmentosa affect a great part of the people of the world. In this kind of illness the other retinal neurons are substantially conserved. Therefore, the use of a biological layer to substitute the damaged retinal photoreceptors is viable.

Studies with the archean *Halobacterium salinarum* discovered that they have a visual protein, bacteriorhodopsin (BR), that resembles the mammalian rhodopsin both structurally and functionally. So it is valuable for the production of an artificial retina.

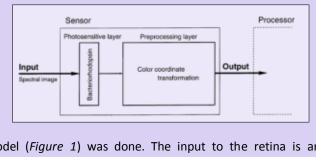


Figure 1. The model of the artificial retina based on bacteriorhodopsin. Modified from [1].

Using BR, a model (*Figure 1*) was done. The input to the retina is an image. It first interacts with the photosensitive layer which converts the input into electrical signals. Then the signals are passed for further transformation to the preprocessing layer. The output of the retina is communicated to the processor. The last two elements simulates the neuronal network.

Despite the model of artificial retina based on BR works, it hasn't been studied yet on animals, they are only laboratory essays.

INDUSTRIAL APPLICATIONS

β-CAROTENE PRODUCTION

Carotenoids are naturally produced pigments that are responsible for the different color of fruits, vegetables and other plants. Among the huge diversity of natural carotenoids we can find β-carotene which functions as an accessory light harvesting pigment, thereby protecting the photosynthetic apparatus against photo damage in all green plants.

Due to its multiple properties has a high demand as a food colouring agent, as an additive to cosmetic, as a source of pro vitamin A and as antioxidant. Nowadays, its highly studied for its antioxidant and immunomodulatory properties as they can be used to prevent cancer. Studies using β-carotene against fibrosarcoma have been found to decrease the levels of many biochemical factors involved in cancer and up-regulate the beneficial ones.

β-carotene is accumulated as a lipid globules in the interthylakoid spaces of the chloroplast in *Dunaliella salina (Figure 3)*, a green algae used for its production. To harvest the pigment the cell has to be lysed and it can be done using a centrifuge or the method of flocculation and surface adsorption. Both methods need a further step of purification.

Figure 3*. Dunaliella salina* rich in β-carotene. From [3]

ECTOINE PRODUCTION

Ectoine is a compatible solute produced by a large number of halophilic microorganisms. It can protect enzymes and nucleic acids against osmotic stress, heat, dryness and freezing. As a result, it increases shelf life and activity of enzymes.

Ectoine protective properties can also be applied to human skin. Cosmetic industry adds ectoine in cosmetics preparations as **moisturizers** for the care of irritated, dry or aged skin.

Topically applied ectoine shows an immunprotective potential on the sun-exposed skin on healthy subjects. The ultra-violet reduction of Langerhans cells has been prevented with ectoine applied before sun exposure.

Industrial process to produce ectoine is based on "bacterial milking". The bacteria are grown to high cell density in a high salt medium, so that they accumulate massive amounts of ectoine in the cytoplasm . Then an osmotic down-shock is applied, and the bacteria respond by secreting most of the ectoine out of the cytosol. Then the compound can be collected by crossflow filtration techniques and purified.

CONCLUSIONS

The studies about the properties of halophiles has revealed their great industrial potential and the multiple applications that can be achieved thanks to our technology. Further studies have to be carried on to exploit at maximum all of the potential application regardless to the field.

Working with these microorganisms has advantages and disadvantages.

Advantage: Halophiles life in rich salt environments which doesn't go under contamination, since the majority of the microorganisms cannot tolerate such high concentrations of salt. This is also useful by the time to harvest material directly from them, because it is only need an osmotic shock.

Disadvantage: The aggressive nature of salts has to be taken in account, as it corrodes the metal. So they need special reactors to grow which will increase the production cost.

Despite the high diversity of halophile and their potential application, the greatest part of the essays are only on the laboratory scale and haven't been demonstrate in real cases, either for the limitations of our current technology or for the field complexity where they could be used..

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