Development of an antibiotic removal system for aquaculture

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Abstract

Food and Agriculture Organization (FAO) studies predict an increase of 40.5% production of aquaculture until 2030 in the world. The effluents from these systems continue to have higher levels of antibiotics, in which about 75% of the drugs fed by fish are excreted in water. Several technologies for removal/degradation of antibiotics in water have been used, such as adsorption, reverse osmosis, liquid-liquid extraction, constructed wetland, electroxidation and photocatalytic technology. In this laboratory scale work, it is proposed to use eutectic solvents in order to remove the drugs using two different approaches: by liquid-liquid extraction and by impregnating the solvent on porous solids. Eutectic solvents based on acid: acid, menthol: acid, thymol: acid, thymol: menthol and quaternary ammonium salt: acid were used to efficiently extract enrofloxacin, chloramphenicol and norfloxacin in aqueous solutions. The solubility of the antibiotics in the solvents was initially determined in order to discard the less soluble solvents. Thus, the worst extractors and the operating conditions were optimized, mainly at the level of the initial concentration of the aqueous antibiotic solution, equilibrium time and amount of solvent used. It was found that the impregnation method has more advantages in relation to liquid-liquid extraction, essentially in terms of reducing the working time and the amount of solvent used, obtaining extraction efficiencies of 82% for enrofloxacin and 84% for norfloxacin using the decanoic acid: dodecanoic acid solvent, and 56% chloramphenicol extraction using the methyltrioctylamonium bromide: decanoic acid solvent. Finally, the possibility of reusing and recycling solvents was verified.

Keywords: aquaculture, antibiotic, eutectic solvent, extraction, impregnation

Introduction

Aquaculture in tanks is based on the use of mechanical and biological filters coupled to a Re-circulated Aquaculture System, RAS. From an environmental point of view, RAS is beneficial, since water is a scarce resource in many regions of the world. In a RAS it is necessary to treat the water continuously to remove the waste excreted by the fish and add oxygen to keep the fish alive. [1] Possible disadvantages of RAS include increased capital investment, increased energy and more qualified management requirements. Although the RAS's are environmentally sound in terms of water conservation, there is the production of a high effluent in solids, nutrients and biochemical oxygen deficiency (BOD), which can have adverse environmental impacts if not properly managed. [2] The growing demand for fish products promoted the intensification of aquaculture production, leading to the wide application of antibiotics for the prevention and treatment of bacterial diseases. Excessive use of antibiotics in aquaculture can result in the presence of residual antibiotics in commercial fish and shellfish products. Unintended consumption of antibiotics can lead to the development of antibiotic resistance in human pathogenic bacteria. [3]

In aquaculture, antibiotics are usually administered in granulated foods, with immersion in water or injection. However, fish do not metabolize antibiotics effectively and much of it is discarded in feces and urine. It is estimated that 75% of antibiotics fed by fish are excreted in the water. These have not always been used responsibly in aquaculture, and the control of their use has not provided an adequate guarantee of risk prevention for humans. Responsible drug use requires

clear instructions from drug manufacturers, proper handling and distribution by dealers, and veterinary supervision in administration by producers. [4]

Globally, China leads aquaculture fish production between 2006 and 2016, in advance of the top fifteen aquaculture producing countries, and Norway in ninth position in 2016, corresponds to the European country with the highest production, according to the FAO. [5]

The known risk of blood diseases and carcinogenic properties of chloramphenicol (CLO), and the absence of safe levels of residues, has led the European Union (EU) to ban it for veterinary use. CLO is also banned in many other countries, including the Unite State of America (USA), Canada, Australia, Japan and China. No maximum limit residual has been established for this antibiotic. Despite this legal prohibition, CLO can be detected in various foods of animal origin, including aquaculture products. It is important to control CLO residues in foods of animal origin and it is necessary to develop sensitive methods for their detection and quantification. [6]

To protect human health from potentially harmful residues of antibiotics, the EU has established safe Maximum Limit Residual (MRL) for substances authorized for veterinary medicinal products in food-producing animals (EU Regulation 2377/90), which describes the procedure for establishing MRLs for medicinal products veterinarians in animal feed. [7]

Between the technologies used in the removal and degradation of antibiotics from wastewater from aquaculture, Cheng et al. (2017) [8] obtained maximum adsorption values with activated carbon for the three antibiotics chloramphenicol, furazolidone and D-cycloserine at the respective values of 32.3, 29.3 and 9.356 mg/g. [8] Yu *et al.* (2020) [9] adsorbed chlortetracycline from aquaculture wastewater with modified lanthanum zeolites, with a removal rate of 98.4%. [9] Dolar *et al.* (2012) [10] studied the application of reverse osmosis and nanofiltration membranes to remove residues of sulfamethoxazole, trimethoprim, ciprofloxacin, dexamethasone and febantel obtained a high level of retention > 95% of all selected antibiotics. [10] Gorito *et al.* (2018) [11] obtained removals of almost 100% for the drugs clarithromycin, fluoxetine and norfluoxetine, using constructed wetlands. Romero-Soto *et al.* (2018) [12] found that the removal of chloramphenicol was 98.7% using electroxidation on a Ti electrode coated with PbO_2 (Ti/PbO_2) in a stirred batch reactor. [12]

Dong *et al.* (2020) [13] studied samples of wastewater that were degraded by the addition of 0.5 g/L of $ZnSnO_3$, and the removal rates of ciprofloxacin and sulfamonomethoxine reached 85.9% and 37.5% after 100 minutes of radiation stimulated solar, respectively. [13] Do *et al.* (2020) [14] tested the photocatalytic degradation process of five antibiotics using TiO_2 nanotubes and introduced them into nanowires using the anodization method, effectively and quickly degrading antibiotics under UV-vis irradiation and obtained more than 95% removal in 20 minutes of doxycycline, sulfamethoxazole, oxytetracycline, lincomycin and sulfamethazine. [14] Leal *et al.* (2016) [15] studied the possibility of applying solar photodegradation to remove 96% of oxytracycline from marine aquaculture waters in 230 minutes. [15]

In 2003, Abbott *et al.* [16] defined a Deep Eutectic Solvent (DES) as eutectic mixtures that exhibit a great depression in the melting temperature of the eutectic point in relation to those of the pure components. In order to identify a DES, its phase diagrams must be known in order to compare the actual temperature depression with that expected for the ideal mixture, and to define composition ranges in which these solvents are in liquid state at operating temperatures. [17] Abbott *et al.* (2003) [16] found that eutectic occurs in a 2: 1 ratio (urea: choline) with a melting temperature of 12 °C, considerably lower than the melting temperature of pure constituent compounds, 302 °C for the choline and 133 °C for urea. This method of forming liquids from mixing solids at room temperature is not limited to choline chloride, but other quaternary ammonium salts exhibit the same property. [16]

Eutectic solvents present enormous potential, not only because they can be easily synthesized, just by mixing and heating, but also allow easy design of their physical and chemical properties only by combination of its constituents. [18]

For a DES to be significantly different from any other eutectic mixture and to give some denotation to the term "deep", a "deep eutectic solvent" must be defined as a

mixture of pure compounds for which the temperature of the eutectic point is below that of the ideal liquid mixture. [17] As the magnitude of the melting point depression depends on the interactions between the components of the mixture, a DES is obtained only for a mixture with a large deviation from ideality (usually by establishing a large network of hydrogen bonds or by the presence of charged compounds). [19]

The different ranges of compositions and temperatures for which a homogeneous liquid phase is available, show that working at a fixed temperature (usually room temperature) and/or fixed composition (eutectic mix compositions and working temperature range) can be selected for the important properties of DES. [19]

Hydrophobic eutectic solvents - DES subclass - based on natural sources were studied by Florindo *et al.* (2019) [19] combining menthol and various natural long-chain fatty acids, as well as other terpenoid compounds, such as menthol and thymol combined with various carboxylic acids. The authors also found that the hydrophobicity of DES depends on the hydrophobicity of the individual compound and DES components formed using a hydrophilic and a hydrophobic compound are not stable in water, as the hydrophilic component leaches the aqueous phase according to its solubility in water. [19] In contrast to DES, these new eutectic solvents showed low viscosities (5-100 cP) and densities below water, as can be evaluated by the density and viscosity at 293.15 K at 353.15 K and under atmospheric pressure, regardless of the water content. [20]

One of the current problems of wastewater is the presence of micropollutants, whose elimination is deficient in wastewater treatment plants, which makes the search for efficient, ecological and cheap water treatment techniques a priority, given the indispensability of this resource in humanity.

This work aims to develop a system for removing antibiotics with recirculation for aquaculture, in particular fish farming, of fish species such as gold fish (*Carassius auratus L.*), European sea bass (*dicentrarchus labrax*), flatfish (*solea senegaleusis*), zebrafish (*Danio rerio*), European eel (*European gloss eel Anguilla*), using eutectic solvents based on terpenes (thymol and menthol), carboxylic acids (octanoic acid, decanoic acid and dodecanoic acid), and quaternary ammonium salt (methyltrioctylamonium bromide) according to the studies obtained by Florindo *et al.* (2019). [21] Neutral and ionic hydrophobic DES's have been developed as extractors of ciprofloxacin, with the purpose of guaranteeing a high percentage of removal of enrofloxacin, chloramphenicol and norfloxacin, and subsequently their reuse in the system, thus preventing the excessive water consumption in fish tanks.

Although the search for DES is fundamental for this work, never less only the term "eutectic solvent" is used to define the mixture of 2 compounds that have a melting temperature below the temperature of each isolated compound not It hasn´t been guaranteed that the melting temperature of these mixtures presents a significant decrease, as in the published studies on the emergence of DES's.

Experimental Section

Materials. Octanoic acid (C8) (purity ≥99%) was purchased from Acros Organics, decanoic acid (C10) (purity ≥99%) was purchased from Alfa Aesar, dodecanoic acid (C12) (purity ≥98%), menthol (Men) (purity ≥95%), thymol (Thy) (purity ≥99%), and methyltrioctylamonium bromide ([N8881] Br) (purity ≥97%) were purchased from Sigma-Aldrich and used as received. All solvents were prepared by weighing the mass required using an analytic laboratory balance Ohaus Adventurer AX223M. The enrofloxacin and norfloxacin were supplied by Alfa Aesar and chloramphenicol was supplied by Sigma-Aldrich, all with high purity (≥98%) and were used as provided. All aqueous solutions were prepared by weighing the mass required using an analytic laboratory balance Mettler Toledo MS205DU, by mixing high purity water (Milli-Q water).

Analytical Methodology. To analyze the presence of antibiotics in aqueous solutions, a Shimadzu UV-1800 spectrophotometer was used.

Experimental Methodologies. Preparation of eutectic solvents. Eutectic solvents were prepared by mixing two components in a glass vial at 50 °C and 500 rpm of stirring speed, until homogeneous liquid mixture (about 1 hour). The molar ratios used to prepare the eutectic solvents used in this work are shown in Table 1.

Table 1 - Molar ratio of the eutectic solvents studied in this work.

Eutectic solvent	Molar ratio
C8: C10	1: 1
C8: C12	3: 1
C10: C12	2: 1
Tim: C8	1: 1
Tim: C10	1: 1
Men: C8	1: 2
Men: C10	1: 2
Men: C12	2: 1
Tim: Men	1: 2
[N8881]Br:C10	1: 2

Solubility of antibiotic in different eutectic solvents. To evaluate the solubility of antibiotic in eutectic solvents, small amounts of antibiotic were added to different solvents (1 mg/2 g), at room temperature (rt), using a water bath. The solutions were vigorously stirred between each addition of antibiotics, for 1 hour and at 400 rpm, where a homogeneous mixture was observed. This procedure was repeated until the saturation point (heterogeneous mixture) was visually detected. Subsequently, it was stirred under the previous conditions, for 24 hours at rt in an ethylene glycol bath, to confirm the saturation, otherwise a new addition was repeated.

Liquid−Liquid Extraction Procedure. Each eutectic solvent was placed in contact with the aqueous stock solution of each antibiotic with concentration of 10 ppm, using a 1: 1 mass ratio. This solution was previously prepared and diluted to prepare standards so that a calibration curve of the antibiotic in Milli-Q water (curve with R^2 > 0.99) could be established. All extractions were performed at room temperature and then left to settle for a minimum of 48 h to ensure complete phase separation. Using a syringe, the aqueous phase was removed and subsequently centrifuged for at least 3 hours at a speed of 6000 rpm, and the antibiotic concentration in the aqueous phase was measured using UV-vis spectrophotometry.

Reuse and recycling of eutectic solvent. To evaluate the reuse of eutectic solvents in subsequent cycles (figure 1), after extraction, the solvent phase was exposed to an aqueous solution with fresh antibiotic, in the same mass ratio (1: 1). This procedure was repeated three times, corresponding to three cycles of extraction.

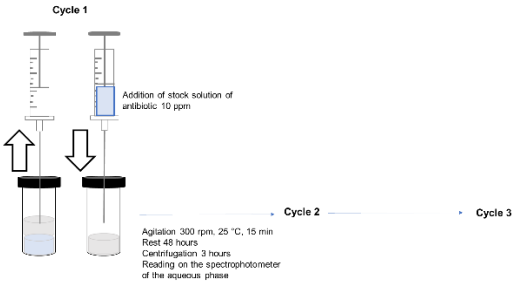

Figure 1 - Scheme for reuse of eutectic solvent by liquid-liquid extraction.

To recycle the eutectic solvent, adsorption with activated carbon was used to remove the antibiotic from the solvent. The phase rich in solvent was mixed for 15 minutes at 300 rpm with 0.1 g of activated carbon in a tube for centrifugation at rt and then centrifuged for 1 hour at 6000 rpm. The clear upper phase was filtered using a hydrophobic polytetrafluoroethylene syringe filter with a 0.45 µm pore size to ensure the complete removal of activated carbon from the eutectic solvent phase. The recycled solvent was transferred to a glass vial for new liquid-liquid extraction and the absorption spectrum was determined.

Solvent impregnation procedure on porous support. A hydrophobic polyvinylidene fluoride (PVDF) membrane with a 0.22 µm pore diameter was weighed before and after impregnation with eutectic solvent, to confirm the mass increase. The membrane was placed in a vacuum system for 1 hour and 2 mL of eutectic solvent was injected into the surface of the membrane, permitting it to impregnate for another 1 hour. The membrane was pierced with a mold to obtain membranes of diameter of 0.2 cm. For extraction, 2 mL of antibiotic stock solution, 524 PVDF membranes impregnated in eutectic solvent and a magnet were added to a glass vial and stirred in water bath at 25 °C and 300 rpm for 15 minutes. The absorption spectrum was read immediately on a UV-vis spectrophotometer.

Step extraction of antibiotic and solvent reuse. After the procedure previously, which corresponds to cycle 1 of extraction, the aqueous antibiotic solution was completely removed from the glass vial, and transferred to a new tube with 524 new membranes (figure 2). The mixture was stirred in a water bath at 25 °C and 300 rpm for 15 minutes and the absorption spectrum was read on the spectrophotometer, corresponding to the cycle 2. The same procedure has been done for the cycle 3.

For the reuse of the eutectic solvent, the solvent impregnation procedure in porous support, which corresponds to the cycle 1, was followed.

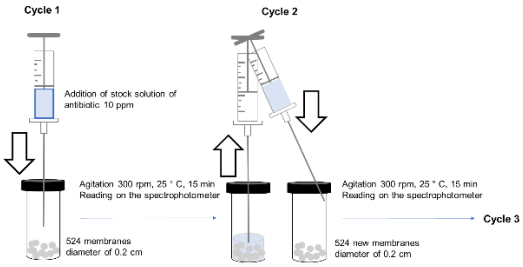

Figure 2 - Scheme of step extraction of the antibiotic by impregnating solvents on a porous support.

Subsequently, the aqueous solution of antibiotic remaining in the glass vial was completely removed with a micropipette, and 2 mL of aqueous solution with fresh antibiotic was added. The solution was stirred in a water bath at 25 °C and 300 rpm for 15 minutes, and the absorption spectrum was determined, corresponding to the cycle 2. The same procedure was repeated for the cycle 3.

Results and Discussion

Solubility of antibiotics in eutectic solvents. The solubility of the 3 antibiotics, enrofloxacin (ENR), chloramphenicol (CLO) and norfloxacin (NOR), in the eutectic solvents is listed in figure 3. This result is the sum of the masses added progressively to the eutectic solvents before precipitation occurs.

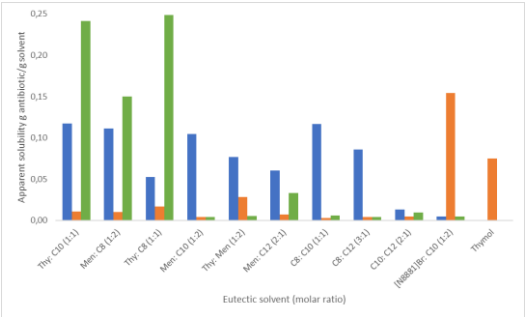

Figure 3 - Apparent solubility of antibiotics in eutectic solvents. All results were measured at 25 ºC and atmospheric pressure, with the exception of Timol, which was measured at 50 ºC. Blue: enrofloxacin. Orange: chloramphenicol. Green: norfloxacin.

The three pharmaceutical compounds have a solubility of the same order of magnitude in the solvents studied and it can also be concluded that enrofloxacin is soluble in a wider range of solvents, contrary to chloramphenicol and norfloxacin. ENR shows better solubility in solvents C8: C10 and Thy: C10 with a similar value of 0.117 g ENR/g solvent and poor solubility in solvent [N8881] Br: C10 with a value of 0.005 g ENR/g solvent. CLO has a maximum solubility for the solvent [N8881] Br: C10 with a value of 0.154 g CLO/g solvent, and a minimum of 0.003 g CLO/g solvent for C8: C10, being insoluble in the acid: acid solvents and menthol: acid. Due to the low values in this class of solvents, the apparent solubility was determined using only Thymol at 50 °C, with a final value of 0.075 g CLO/g solvent; NOR has a maximum solubility for Thy: C8 and Thy: C10 with a value of 0.249 and 0.242 g NOR/g solvent, respectively, and a minimum of 0.004 g NOR/g solvent for C8: C12. It seems, that the solvents in which each drug is more soluble, will be the ones that will have the best extractions efficiency. However, in the impregnation of solvent in porous support, this assumption is only confirmed in the case of CLO: the solvent [N8881] Br: C10 has better extraction efficiencies. In the case of ENR and NOR the same solvent C10: C12, with very low solubilities, presents better extraction percentages.

Optimization of experimental extraction conditions. Several aspects of the extractions had to be optimized, in order to improve the extraction procedure, decrease the contamination of the phases and increase the extraction of drugs in eutectic solvents. Thus, following the work done by Florindo *et al.* (2019) [21], a stirring speed of 300 rpm was used in this work; 15 minutes of stirring between the eutectic solvent and the aqueous solution of antibiotic; resting time of 48 hours, so that there is complete separation of organic and aqueous phases; centrifugation for at least 3 hours of the aqueous phase at a speed of 6000 rpm.

There is high contamination of solvents in the aqueous phase, mainly in the zone below 300 nm, coinciding with the wavelengths of the 3 antibiotics. The solvents Thymol: acid and Thymol: Menthol although hydrophobic, show some solubility in water contaminating it, through the presence of the bands of the aromatic group of thymol, and the bands of acids very pronounced in the area of wavelengths below 300 rpm, again coinciding with the wavelength zone of the 3

antibiotics. In this way, these solvents were discarded in subsequent tests.

Initial concentration of antibiotic stock solution. It is noted that the extraction efficiencies for the three antibiotics are high, above 90%. As mentioned, the fact that there are peaks of the solvent in the UV-vis region where pharmaceutical compounds absorb can indirectly contribute to these high values and, therefore, these values may not be as high. On the other hand, only for enrofloxacin, concentrations adjusted to the calibration curve, less than 5 ppm, were obtained, a concentration lower than that of the analyzed stock solution. The same is not true for chloramphenicol and norfloxacin, that is, the contamination of the eutectic solvent is more relevant for CLO and NOR.

The best results were obtained for a concentration of 10 ppm of antibiotic stock solution. The best extraction efficiencies were obtained for the case of norfloxacin, which in general are above 98% for all solvents analyzed.

Reuse and recycling of the eutectic solvent. The reuse of the solvent by liquid-liquid extraction works better only for enrofloxacin 10 ppm in 3 cycles of extraction and recycling of the solvent C10: C12, as shown in figure 4.

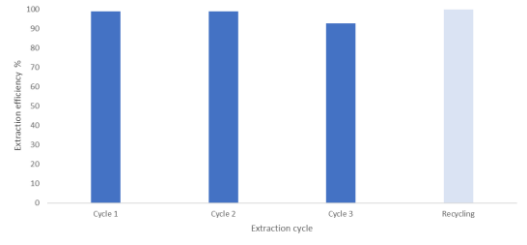

Figure 4 - Graph of reuse and recycling of C10: C12 by the liquid-liquid extraction obtained for enrofloxacin with a concentration of 10 ppm. Dark blue: extraction cycles 1, 2 and 3 in solvent reuse; Light blue: solvent recycling.

The extraction efficiency decreases only slightly, presenting a value of 98.7% for cycle 1; 98.9% for cycle 2 and 92.7% for cycle 3. After recycling the solvent C10: C12, it was possible to maintain the extraction efficiency obtained initially for enrofloxacin, in the amount of 99.7%. Thus, the feasibility of the reuse and recycling efficiency of this extractant can be concluded.

Optimization of the conditions of the solvent impregnation method in the porous support.

Initially, the solubility of the solvent C10: C12 impregnated in the porous support in ultrapure water was tested and it was verified that there is no passage of decanoic or dodecanoic acids into water, due to the absence of characteristic bands of these acids in the UV-vis spectrum (length of less than 270 nm). The solvent remains impregnated to the support, probably due to having a greater affinity for the hydrophobic support than for water.

Another situation evaluated was whether the increase in the amount from 34 to 51 membranes, both 0.5 cm in diameter, would have an influence on the extraction efficiency. In principle, an increase in the percentage of extraction would be expected due to the increase in the solvent quantity, but on the contrary, there is practically no influence on the extraction when increasing the quantity of membranes. In fact, the maximum extraction was obtained for 34 membranes of diameter 0,5 cm.

The size of the impregnated phase is an essential factor in the extraction with microphases, because the greater the surface area of the impregnated phase, the faster and more efficiently extraction will occur. To optimize the size of the impregnated porous phase, it was found that 34 membranes of 0.5 cm in diameter contain approximately the same mass of solvent as 524 membranes of 0.2 cm in diameter, making it possible to increase the percentage of norfloxacin extraction, using the same solvent C10: C12, from 50% (34 membranes) to about 79% (524 membranes) when the size of the impregnated porous phase is reduced, keeping the mass of solvent used constant, as shown in figure 5.

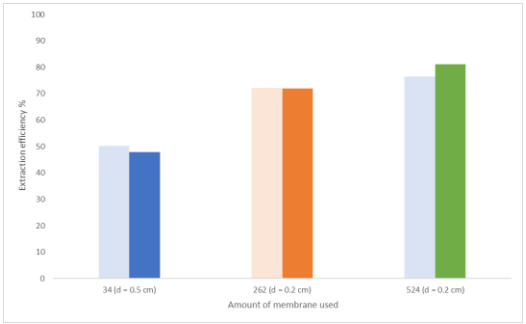

Figure 5 - Norfloxacin extraction efficiency chart, using 34 membranes with 0.5 cm diameter and 262 and 524 with 0.2 cm diameter, impregnated in C10: C12. Light blue: test 1 for 34 membranes; Dark blue: test 2 for 34

membranes; Light orange: test 1 for 262 membranes; Dark orange: test 2 for 262 membranes; Light green: test 1 for 524 membranes; Dark green: test 2 for 524 membranes.

Antibiotic step extraction and reuse of eutectic solvent. In step extraction, it is possible to find how many extraction cycles are necessary to achieve a complete removal of the antibiotic from the aqueous phase by the eutectic solvent. Thus, for the optimized conditions, 2 extraction cycles are sufficient to allow the complete extraction of antibiotics in 10 ppm aqueous solution, with the respective eutectic solvents, that is, C10: C12 for enrofloxacin and norfloxacin and [N8881] Br: C10 for chloramphenicol.

The reuse of solvents in 3 extraction cycles (figure 6), it is possible to consider the cycles 1 (82.5% ENR and 73.2% NOR) and cycle 2 (66.5% ENR and 46.5% NOR) when reusing C10: C12. The solvent [N8881] Br: C10, which allows the extraction with higher efficiency of chloramphenicol, has a great capacity for reuse, since in 5 consecutive cycles it manages to maintain the extraction value at about 60%, decreasing only in cycle 6 to 36,4%.

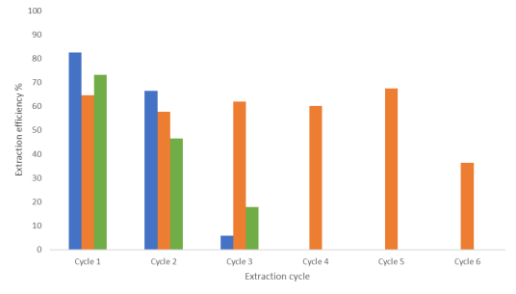

Figure 6 - Graph of efficiency of antibiotic extraction in the reuse of impregnated solvent in support. Blue: enrofloxacin; Orange: chloramphenicol; Green: norfloxacin.

Concentration of the initial stock solution. In order to compare the laboratory tests of a real situation of application to the discharge of aquaculture water to remove antibiotics, experiments were carried out reducing the concentration of the antibiotic stock solution from 10 ppm to 2 ppm. The results obtained under optimized conditions in solvent C10: C12, reveal a loss of extraction efficiency to 56% in removal of norfloxacin, that is, a greater mass of impregnated solvent is needed to extract drug from this type of system for aquaculture, where the concentration of antibiotic in the discharge zone is in the order of ng/mL.

Conclusion

The solubility of antibiotics in eutectic solvents is the most relevant initial parameter in order to discard solvents with less efficient extraction. Thus, it is concluded that in general the solvents with greater solubility in drugs, consequently extract better.

Using the optimized liquid-liquid extraction method, extraction percentages are obtained with better results for norfloxacin in C10: C12, although these results are overestimated since proved effective passage of solvent into the aqueous phase.

The solvent impregnation method in porous solids has advantages over liquid-liquid extraction, namely: reducing the experimental work time from about 52 hours to 3 hours; reducing the mass of solvent used from 2 grams to about 0.105 grams. Optimizing the conditions of this method, it is concluded that the best extractors for enrofloxacin and norfloxacin, and chloramphenicol were C10: C12 and [N8881] Br: C10, respectively.

The application of the results obtained by solvent impregnation in support for the real context, must be properly dimensioned, since it was concluded that the decrease in the concentration of antibiotics in aqueous solutions translates into loss of extraction efficiency. Thus, a greater amount of solvent is needed to extract antibiotic solutions with low concentrations.

It is possible to reuse and recycle the solvents by liquid-liquid extraction, with the best results for the case of aqueous solutions of enrofloxacin, reusing the solvent C10: C12 in 3 cycles and the recycling of the same solvent showed an efficiency as an initial extraction. By impregnating the solvent in the membrane, 2 cycles are sufficient to completely extract the 3 antibiotics, being a great result in the extraction in stages. The solvent [N8881] Br: C10 showed great capacity for reuse in 5 cycles in the case of chloramphenicol, although of all the solvents this is the least sustainable solvent.

It is important to study the solid-liquid equilibrium graphs of the solvents before application in the laboratory, as proof that the presence of a DES is effectively

guaranteed, as evidenced by the great depressing of the melting temperature, compared to the melting temperatures of each compound individually.

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