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Antibiotics in swine husbandry effluents: Laying the foundations for their efficient removal with a biocompatible ionic liquid

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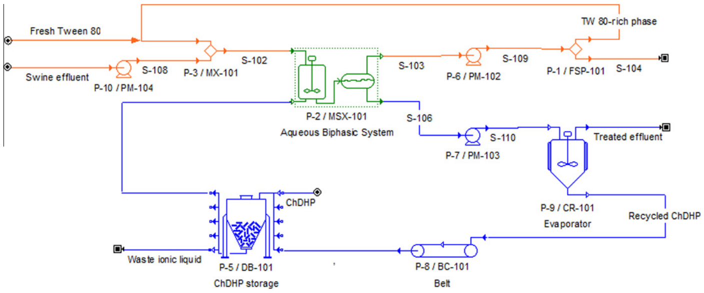
HIGHLIGHTS

- Choline-based ABS was successfully proposed to remove antibiotics from effluents.
- ChDHP turned out to efficiently salt out aqueous solutions of non-ionic surfactants.
- Extraction values higher than 96% were obtained for tetracycline and oxytetracycline.
- Artificial urine and real waste swine effluent did not alter the biphasic region.

GRAPHICAL ABSTRACT



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ABSTRACT

The presence of emerging contaminants such as antibiotics is currently urging the search of new techniques to efficiently remove them from the environment. In this work, the exceptional ability of a cholinium-based ionic liquid to salt out aqueous solutions of non-ionic surfactants containing tetracycline and oxytetracycline has been demonstrated. The deep characterization of the phase diagrams has allowed implementing the process with an antibiotic-polluted effluent, reaching extraction levels higher than 96%. The viability of the proposed strategy has been checked in a urine-type effluent and real swine wastewater stream, observing that no important alterations are detected in the phase segregation capacity, and values of antibiotics removal over 70% are reached. The number of cycles that both the ionic liquid and the surfactant can be reused demonstrates the potential of the proposed process to be applied at a real swine wastewater.

1. Introduction

The use of antibiotics has been rapidly extended in the last decades due to their capacity to treat manifold infectious illnesses [1], thus leading to a massive increase of their presence in the environment. More specifically, their indiscriminate use to prevent diseases in livestock has contributed to christen them as emerging contaminants [2]. Different antibiotics have been employed in ranching such as β-lactams, macrolides, sulfonamides, fluorochinolones, polyethers and tetracyclines. Among them, tetracyclines have been detected worldwide in both manure samples and wastewater effluents from animal husbandry [3–5]. 50–80% of these compounds are not metabolized and are thus excreted through animal urine and feces [6]. Due to this, manure can be considered the main source of contamination and different studies have illustrated the dimensions of this problem. In this sense, the concentrations of tetracycline in cattle, poultry and pig manure reached levels of 1.65, 15.62 and 16.56 ppm, respectively [7]. These figures underscore the importance of this problem in pig farms, and these pollutants have been detected both in aquifers and soils in areas of Spain, USA and China [7,8]. Therefore, since pig manure

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is considered as a natural fertilizer, the presence of these emerging contaminants is gaining gargantuan dimensions, as they are not restricted to the surroundings of swine facilities.

Typically, these pollutants are advected in the environment and can be adsorbed to suspended matter or sediments, so the scientific community is urged to hunt for competitive alternatives to remove them from the environment. Only a few methods have been explored for the treatment of tetracycline-based antibiotics: biodegradation and oxidation [9–10]. However, the high operating costs associated with the latter and the complexity of this specific kind of wastewater could hinder the achievement of high remediation levels. For example, different authors have reported the presence of detergents coming from the cleaning operations in swine houses [11]. In this context, the existence of tensioactive compounds could be used as an opportunity, as they may facilitate the existence of biphasic media. Thus, liquid–liquid extraction springs up as an appealing alternative to remove antibiotics from effluents. More specifically, aqueous biphasic systems (ABS) consist of the addition of salting out agents to aqueous mixtures of hydrophilic compounds (e.g. polymers or surfactants) that promote a decreased water solubility of the latter, up to reach phase segregation [12]. These systems have been proposed for the separation of a wide range of compounds with industrial interest, such as monomers [13], enzymes [14], organic low-molecular weight molecules [15], metal ions [16], antioxidants [17] or alkaloids [18].

During the last years, the tunability of ionic liquids, salts that are liquid near room temperature, has contributed to boost their use for the implementation of new types of ABS. In this sense, our group has recently demonstrated the ability of these salts to trigger phase disengagement in aqueous solutions of surfactants [19]. In this work, we concluded that the use of more hydrophilic ionic liquids leads to greater immiscibility regions. Therefore, a choline-based ionic liquid would fulfil this requirement and, at the same time it involves environmental benefits, as this cation has been highlighted as a biocompatible one. Regarding this, since cholinium chloride is an essential nutrient, its biodegradability and low toxicity has already been demonstrated [20–21]. In this vein, we have recently concluded the suitability of this environmentally benign ionic liquid to promote segregation in aqueous solutions of non-ionic surfactants [22]. Following this research line, in the present study we hypothesize that the use of a more hydrophilic anion may entail bigger immiscibility areas. Hence, the presence of dihydrogen-phosphate anion instead of chloride anion would make the ionic liquid more prone to establish hydrogen bonds with water molecules (predicted log P of -1.02 and 0.61, respectively) [23].

Bearing in mind the abovementioned, the suitability of choline dihydrogen phosphate (ChDHP) to salt out antibiotics from swine waste effluents containing non-ionic surfactants will be tackled. Tween 80 and Tween 20 have been cherry-picked as model non-ionic surfactants with widespread application in food, biotechnological and domestic sectors [22]. Hence, the experimental determination of the phase diagrams for ternary systems composed of Tween surfactant, water and ChDHP will be the foundations to build a green, efficient and low cost extraction strategy with a real effluent from swine farms.

2. Experimental methods

2.1. Materials

The ionic liquid trimethyl hydroxyethyl ammonium dihydrogen phosphate (ChDHP, >98%) was purchased from IoLiTec. It was dried under vacuum conditions ($2 \cdot 10^{-1}$ atm) and moderate temperature (50 °C) for several days. Afterwards, it was maintained in bottles under inert atmosphere until use. The surfactants Tween 20 and Tween 80 were supplied by Sigma–Aldrich. The same company provided the antibiotics tetracycline ($\geqslant$98%) and oxytetracycline ($\geqslant$95%). Synthetic urine was carried out in accordance with the recipe reported by Freire and coworkers (1.2 g/L of urea and 4 g/L of NaCl) [29]. Real wastewater was collected from a Galician (NW Spain) swine farm in September 2015.

2.2. Aqueous biphasic systems

The definition of the immiscibility regions was experimentally carried out by visually detecting turbidity changes in ternary mixtures composed of water, surfactant and ionic liquid. In brief, water was added to binary mixtures of surfactant (Tween 20 or Tween 80) and ChDHP with known compositions until the solids are solubilized. The composition of this ternary mixture was ascertained by weighing in an analytical Sartorius Cubis MSA balance (125P-100-DA, $\pm 10^{-5}$ g). Afterwards, more water was added until a transparent solution was observed, and again this composition was determined by weighing [19]. The area between these two compositions is the liquid–liquid immiscibility region. All these experiments were carried out under constant stirring in a thermostated glass cell to control the temperature at 25, 40, 50 and 60 °C by means of a F200 ASL digital thermometer.

For the tie-line determination, a ternary mixture belonging to the immiscibility region with known composition was shaken for one hour and left to settle in a decanter for 48 h. Then, both phases were separated and their composition was ascertained by density and refractive indices measurements. These compositions were inferred from a polynomial fitting of these two physical properties obtained from different ternary mixtures belonging to the binodal curve. Densities and refractive indices were measured in an Anton Paar DSA-48 digital vibrating tube densimeter ($\pm 2 \cdot 10^{-4}$ g/mL), and a Dr. Kernchen ABBEMAT WR refractometer ($\pm 4 \cdot 10^{-5}$), respectively.

2.3. Antibiotics extraction

The extraction experiments were always carried out in complete darkness, and appropriate dilutions were applied to ease antibiotics determination. The demonstration of the ionic liquid and surfactant recyclability was performed by reusing them. On the one hand, the ionic liquid was introduced in a rotatory evaporator at 70 °C under vacuum ($9 \cdot 10^{-2}$ atm) as a prior step to reusing it in the following cycle. On the other hand, 1.75 mL of swine wastewater containing oxytetracycline and tetracycline concentrations around 100 ppm were vigorously mixed (1 h) with the corresponding surfactant and ionic liquid concentration. The mixtures were left to settle in a decanter for 2 days prior to phase separation. Both the surfactant and the recovered ionic liquid were added to a new sample containing swine wastewater, and this procedure was repeated up to complete 5 cycles. The concentration of antibiotics in each phase was ascertained by HPLC analysis.

2.4. Antibiotics determination

Tetracycline and oxytetracycline were analyzed by means of HPLC–DAD at 274 and 272 nm, respectively (Agilent 1260 infinity), equipped with a Kinetex Biphenyl column (4.6 × 150 mm, internal diameter 5 μm). All samples were passed through a 0.45 μm PDVF filter prior to injection in order to avoid the presence of solids. 10 μL of sample were eluted in gradient mode at a flow rate of 1 mL/min, using a mixture water:methanol:ammonium formate as follows: 85:10:5 to 50:45:5 in 5 min. From 9 to 14 min, the concentration is changed to 25:70:5, maintaining this ratio for 2 additional minutes. Retention times for tetracycline and