nomilin has been the main cause of delayed bitterness of citrus fruits.

In this study, the selective removal of limonin from orange juice by batch adsorption to different microstructured resins was investigated. Since the removal of reducing sugars, pigments and vitamin C may also occur, the eventual adsorption of these compounds was also investigated. The following synthetic neutral resins, from Rhom and Haas, were tested: Amberlite XAD-8, XAD-16N and XAD-1600. The experiments were performed in a microscale, using 24 wells microplates. Different amounts of these sorbents, 0.5—25 g L^{-1} were added to 2 mL Navel orange juice (pH 3—3.5) previously centrifuged at 4000 rpm.

The highest adsorption efficiency for the bitter compound was observed when the synthetic neutral resin, Amberlite XAD-16N, was used, followed by XAD-18. The adsorption of sugars, pigments and vitamin C to the resins were also evaluated. Both Freundlich and Langmuir isotherm models were fitted to the experimental data of adsorption of limonin to the resins used. The uptake of limonin, throughout the time, to the sorbents XAD-16N, XAD-18 and XAD-1600 was tested.

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2.5.38

Improvement of specific monoclonal antibody (mAb) activity by reduction of the mAb heterogeneity using continuous chromatography (MCSGP)

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Monoclonal antibodies (mAbs) are among the most important therapeutic proteins in development and production by the biopharmaceutical industry.

However, mAbs actually represent mixtures of different molecules themselves: due to post-translational modifications and degradative processes, the mAbs display a large heterogeneity. Different glycoforms, deamidated variants, lysine variants, etc. have been reported. Although it is known that the variants may have different biological activities, a separation of the variants has not been performed on a preparative scale yet due to a lack of suitable technologies. It has been shown that with multicolumn countercurrent solvent gradient purification it is possible to separate monoclonal antibody charge variants with high yield and purity using a cost-effective cation exchange stationary phase. Thus, by means of MCSGP the specific activity of the drug may be increased by removal of less active charge variants. This aspect may be of particular interest with respect to life cycle management of mAb-based drugs. In the work presented, the variant separation using MCSGP is shown for a commercially available mAb including process development, modeling and experimental data.

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2.5.39

Sulphate-reducing bacteria from mining environments for metals bioremediation

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Toxic heavy metals and metalloids constitute an international pollution problem that not only impacts public health but also is of environmental and economic importance. Several conventional treatment technologies for removing metals are available. These techniques, based on chemical methods of neutralisation and precipitation, even though quick and effective, present several disadvantages, such as the need for building additional treatment plants, the high cost of the chemical reagents used and the generation of an important volume of sludge which need further treatment.

Prokaryotes with physiological activity of sulphate reduction are found in several environmental sites containing metals and these microorganisms have developed several different strategies for detoxification and resistance to toxic elements that are potentially useful for bioremediation. Since sulphate-reducing bacteria (SRB) are found in a large number of contaminated sites containing toxic metals, it is apparent that these organisms have a functional defence system that enables them to persist and even grow under metal stress. The enzymatic metal reduction by SRB offers an alternative to chemical processes to remediate environments containing metals.

Acid mine drainage (AMD) is one of the most important source of heavy metal environmental pollution. AMD is characterised by its high acidity ($\leq$3), high concentration of metals (e.g. Cu, Fe, Zn, A1, Pb, As and Cd) and high concentration of dissolved sulphates ($\geq$3000 ppm). Taking advantage of the fact that SRB are present in these mining environments, several samples were collected from S. Domingos abandoned mine (Portugal) and screened for their ability to sulphate reduction, metal resistance and bioremediation.

In this context, the most promising consortium of SRB (SRB no. 6) was grown in matrices column reactors, using lactate and ethanol as an electron donor source for the production of H_2S from sulphates. Metal resistance was tested in batch using different metal concentration for Cu, Zn and Fe. The results obtained for this SRB inoculum, showed 97% of sulphate reduction (3750—350 mg/l) in the reactors, and a metal tolerance to Fe, Cu and Zn, at concentrations until 260 mg/l, 40 mg/l and 80 mg/l, respectively, in the batch assays, being observed a sulphate reduction (2000—0 mg/l) and a significant metals concentration decrease in the growth medium. Further studies are being carried out to apply this mining inoculum for the design of reactors for AMD bioremediation.

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