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Lead-binding capacity of calcium pectates with different molecular weight

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ABSTRACT

Nowadays, heavy metal contamination of environment is considered as a serious threat to public health because of toxicity of these pollutants and the lack of effective materials with metal-binding properties. Some biopolymers such as pectins were proposed for removal of metal ions from industrial water disposals. Chemical structure of pectins is quite variable and substantially affects their metal binding properties. In this work, relationship between molecular weight and Pb(II)-binding capacity of calcium pectates was investigated in a batch sorption system. The results showed that all pectate samples are able to form complexes with Pb(II) ions. The effects of contact time, pH of the media and equilibrium metal concentration on metal-binding process were tested in experiments. The equilibrium time min required for uptake of Pb(II) by pectate compounds was found to be 60 min. Langmuir and Freundlich models were applied for description of interactions between pectates and metal ions. Binding capacity of low molecular pectate was highest among all the samples tested. Langmuir model was figured out to be the best fit within the whole range of pH values. These results demonstrate that calcium pectate with low molecular weight is more promising agent for elimination of Pb(II) ions from contaminated wastewaters.

1. Introduction

Fast growth of industrial activity and technological progress worldwide within past decades resulted in a vast release of metal pollutants into environment. Among them, heavy metals are considered as highly dangerous substances to human beings because even at low concentrations they exert substantial toxicity, incremental accumulation in the food chain, and stable persistence in environment [1]. That is why, today, removal of heavy metals from water reservoirs has gotten a great attention of toxicologists and environmental researchers. Pb(II) is the one of the most commonly used heavy metals in industry. Thus, Pb(II) level in water and soils in industrial areas is very high, and this poses a threat to health of people, in particular, children. Several methods for elimination of heavy metals from aqueous solutions are in use including filtration, chemical precipitation, adsorption, electrodeposition, and membrane systems [2–4]. But all of them are either very expensive or less effective. Numerous studies showed that biosorption is more effective method for the removal of heavy metal ions [5]. Such natural biopolymers as carrageenans, chitins, alginates as well as pectins, possess significant metal binding capacity. They were proposed for removal of the metal ions from water reservoirs [6–8].

Pectins are the ionic plant polysaccharides consisting of the linear (l-4)-linked chains of galacturonate units [9,10]. Natural pectins function as cementing compounds in a cell wall of all terrestrial plants and seagrasses [11]. Three-dimensional structure of these biopolymers provides their effective interaction with metal ions. Therefore, pectins are considered as quite promising and available biopolymers with metal-binding activity [12]. The main mechanism of interaction between pectin molecules and metal ions is proposed to be a formation of specific junction zones termed “egg-boxes” [13]. This mechanism supposes that several free carboxyl groups of galacturonic units located on the biopolymer chain act as the active sites and form links with only one metal ion. Majority of galacturonic residues in natural pectins are esterified with methyl radical and this prevents their interaction with metals ions [14]. That is why low esterified pectins are considered as more effective metal-binding agents [7]. Also, differences in chemical composition and physicochemical properties such as molecular weight, degree of esterification, dispersion, uronic acid content, presence of Ca(II) ions also alter metal-binding capacity of pectins. Thus, it is impossible to predict metal-binding capacity of different samples of commercially available pectin compounds and use them for

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purification of water reservoirs [15]. Polysaccharides with homogenous structure may be more favorable for such purposes. Controlled pectin degradation producing low molecular pectin fractions is believed to be a promising way for successful use of these biopolymers for removal of heavy metals. Besides, low molecular pectins can change their orientation and form the higher number of bonds with metal ions. Homogenous low molecular pectin fractions can be standardized as it contains oligomers with the same degree of polymerization. Ca(II) ions are known to mitigate metal-binding activity as they help to form correct "egg-box" structure and they can be easily substituted with Pb(II) ions due to the very low affinity [16].

Nowadays, relationship between metal-binding capacity of pectins and their degree of esterification is very well studied [8]. But the influence of molecular weight and degree of polymerization on the metal uptake by pectins is not known yet. The main goal of the present study was synthesis of homogenous calcium pectate samples with different molecular weight and estimation of their Pb(II)-binding capacity. The equilibrium of sorption of Pb(II) ions by pectate compounds was studied. The influence of experimental conditions such as pH, agitation period, and equilibrium concentrations on metal-binding capacity was analyzed. The Langmuir and Freundlich equations were used to fit the equilibrium isotherms. The results will be useful for application of system design purposed for treatment of industrial wastewaters.

2. Material and methods

2.1. Materials

Commercial high-esterified citrus pectin with claimed molecular weight about 400 kDa with no additives was purchased from Herbstreith & Fox Corporate Group, Neuenbürg/Württ, Germany. According to the manufacturer's claims the degree of esterification of this pectin was approximately 60.0%. The pectin also contained no acetyl or amide groups. All other chemicals used throughout the study were of the highest quality available.

Method of alkali de-esterification was applied for preparation of the low esterified pectin samples [17]. Preparation of the calcium pectate beads was performed as follows: 100 g of hydrolyzed low-esterified pectin fractions were suspended in 500 ml 70% ethanol solution. Then 22.6 g $CaCl_2 \cdot H_2O$ preliminary dissolved in 100 ml of 70% ethanol solution was gradually added and incubated for 60 min with constant stirring. After that calcium pectate beads were carefully filtered through a glass filter, rinsed with 800 ml of 70% ethanol solution, and then dried at 60 °C until constant weight.

2.2. Pectin hydrolysis

The low-esterified pectin sample was hydrolyzed under controlled unchanged conditions with a constant temperature of solution 90 ± 0.5 °C.

Velocity of the pectin de-polymerization was estimated by the use of the method of step-by-step hydrolysis as follows. 15 g low esterified pectin sample was placed into a reaction tube and then 300 ml 0.5 M HCl solution was added with constant stirring. Reaction tube was placed on a heater and temperature was strictly controlled throughout the whole period of agitation. 30 min after the start of agitation period, 15 ml sample was taken out of the reactive tube and subjected to chemical analysis. The contents of neutral sugars, anhydrogalacturonic acid as well as intrinsic viscosity of the sample were determined. Then, the samples of hydrolyzed pectin were collected from the reaction tube every hour and analyzed for their chemical properties. After each period of agitation, concentration of pectin fractions with different molecular weight in solution was determined. Fractions of low molecular pectins were separated using ultrafiltration membranes. Amount of the pectin was estimated on the base of anhydrogalacturonic acid content in each fraction. Molecular weight distribution of pectin of each fraction was confirmed by HPLC method as described below. Deceleration of the degradation process was achieved by lowering temperature of the reactive system to 0 °C by placing ice cubes into the water-bath. For separation of the liquid pectin phase a centrifuging at 4000 rpm for 30 min was used. The samples were rinsed with 0.5 M HCl and then suspended with ten-fold volume of this acid with following centrifuging and removal of liquid phase containing soluble products of pectin degradation by filtration.

Three fractions of hydrolyzed pectin were supposed to be obtained i.e. fraction A – up to 48.0 kDa, fraction B – from 12.0 to 20.0 kDa, and fraction C – from 5 to 12 kDa. Synthesis of each fraction required a certain period of agitation in acidic media. Duration of each period was determined experimentally as described before. For precise separation of different pectin fraction the ultrafiltration membranes with determined pore size were used.

2.3. Pectin analysis

Degree of esterification and anhydrogalacturonic acid content of the pectin samples were determined using conductometric titration method [18]. Samples of dried pectin (0.05 g) were dissolved in 50 ml ultrapure water in closed flasks and remained in a drying cabinet for 12–15 h at 50 °C. These solutions were titrated with 0.05 M NaOH until pH of the solution reaches 8.5 ± 0.2 controlled with a digital pH meter. The volume of NaOH used was referred as V_1. 10 ml 0.5 M NaOH was added for 30 min at 30 °C in a drying cabinet to induce the process of saponification. The solution was then neutralized by addition of the same volume of 0.5 M HCl. The excess of HCl was titrated with 0.05 M NaOH, and the result was referred as the final volume (V_2).

The degree of esterification was calculated as

$$DE\,(\%) = \left(\frac{V_2}{V_1 + V_2}\right) \cdot 100.$$

Dissolved pectin samples were sedimented by addition of double volume of 96% ethanol solution.

The total carbohydrate content of each pectin sample was calculated in accordance to the phenol-sulfuric method with D-galactose used as a standard [19]. Calculation of the neutral sugar content was performed by taking anhydrogalacturonic acid amount determined by reaction with m-hydroxyohenyl from the total carbohydrate content.

2.4. Molecular mass distribution

Molecular weight of the pectin fractions used in the study was determined using HPLC system equipped with CBM-20A Prominence communications bus module, DGU-20A5 degasser, CTO-20A column oven and RID-20A refractive index detector, and LC-20AD system controller (Shimadzu, Kyoto, Japan). Acquisition of the data values and their analysis were executed using the LC Solution Version 1.25 with GPC option.

Analytical measurements of the molecular weight distribution of pectins were made using high performance size exclusion chromatography on SB-804HQ (8 mm × 300 mm, Shodex, Shanghai, exclusion limits 1 × 106 g/mol, pore size maximum2000). For the standards, the series of pullulan (Fluka, Belgium) were used for calibration as follows: P-1 (MW = 1.32 kDa), P-5 (MW = 5.9 kDa), P-10 (MW = 11.8 kDa), P-20 (MW = 22.8 kDa), P-50 (MW = 47.3 kDa), P-100 (MW = 112.0 kDa), P-200 (MW = 212.0 kDa), P-400 (MW = 404.0 kDa), P-800 (MW = 788.0 kDa). All reagents and mobile phases were prepared using ultrapure water obtained on