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Direct capture of product from fermentation broth using a cell-repelling ion exchanger

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Abstract

A new technique for treating anion exchangers has been proposed allowing direct capture of the fermentation product, shikimic acid directly from the cell-containing fermentation broth. A layer of hydrophilic polymer, poly(acrylic acid) (PAA) has been physically adsorbed on the anion exchanger followed by a covalent cross-linking of PAA. The PAA layer is penetrable for small molecules despite being negatively charged as PAA is, but the polymer layer repels large negatively charged structures like cell debris and cells preventing them from adsorption to the chromatographic matrix. The binding capacity for pure shikimic was about 81 mg/ml adsorbent for both cross-linked PAA–Amberlite and native Amberlite in the fluidized mode of column operation. Binding capacity dropped to 17 and 15 mg per ml adsorbent, respectively, when using filtrated fermentation broth and to about 10 mg/ml adsorbent for cross-linked PAA–Amberlite when using directly the fermentation broth containing cells. Native Amberlite cannot be used for the direct capture of shikimic acid due to the immediate clogging of the column and the collapse of the expanded bed. The cross-linked PAA–Amberlite was used repeatedly for the direct adsorption of shikimic acid from the industrial fermentation broth.

Keywords: Fermentation broth; Stationary phases, LC; Ion exchangers; Shikimic acid; Poly(acrylic acid)

1. Introduction

Fermentative production of low-molecular-mass chemical organic products is an attractive alternative to traditional chemical processes. The fermentation products are synthesized in a more environmentally friendly way with less toxic by-products as compared to the chemical synthesis. One of the main problems to be tackled in the fermentation industry is the capture of the product from a rather dilute fermentation broth, e.g. from a complex mixture containing cells, cell debris and numerous soluble proteins and other macromolecular cell components.

The first stage of the purification of non-volatile chemicals from dilute aqueous solutions is a capture of the product by adsorbing it to an insoluble sorbent. The product is then eluted as a much more

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concentrated solution and subjected to further purification. The direct capture of the product from the fermentation broth is hindered by the presence of cells, cell debris, proteins and nucleic acids in the broth. All these macromolecular structures have a strong tendency to bind to the adsorbents resulting in the decreased availability of the adsorbent for the target product, clogging problems and severe problems with the regeneration of the adsorbents for the next application cycle [1–6]. Traditionally this problem is overcome by separation of the cells, pretreatment of the broth to achieve protein coagulation and separation of the coagulated proteins before capture of the target product.

The objective of the work reported in this paper was to develop adsorbents for the direct capture of products from fermentation broths. This objective is aimed by the development of adsorbents with an attached layer of hydrophilic polymer, poly(acrylic acid) (PAA). The polymer layer is penetrable for small molecules but it repels large structures and cells preventing them from adsorption to the chromatographic matrix. The hydrophilic nature of the polymer layer shields the absorbing structures and thereby prevents the adsorption of the above mentioned species on the modified adsorbent.

2. Materials and methods

2.1. Materials

Amberlite IRA-401 Cl and Amberlite IRA-458 Cl were obtained from BDH (Toronto, Canada). Shikimic acid was a gift from BioGaia Fermentation (Lund, Sweden). PAA with molecular masses of 170 000 and 2100, was from Fluka (Buchs, Switzerland). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 1,6-hexamethylendiamine were delivered by Sigma (St. Louis, MO, USA). Acetic acid came from Merck (Darmstadt, Germany) and poly(*N,N*-dimethyl-3,5-dimethylenepiperidinium chloride) (PDDPC) from Acros Organics (NJ, USA). Bakers yeast in the form of pressed blocks was from a local supplier. The buffer salts were of the best quality available.

2.2. Preparation of PAA-treated Amberlite IRA-401 and IRA-458

Amberlite IRA-401 (Cl) and IRA-458 (Cl) were pretreated according to the manufacturer's recommendations. The resin (6 ml) was suspended in deionized water, packed in a column (15×1 cm I.D.) and washed with deionized water at a linear flow-rate of 300 cm/h. About four bed volumes of 4% NaOH were then passed through the resin to replace Cl^- ions on the resin with OH groups, and finally the column was rinsed in an upward mode with water until the pH in the effluent was around 7.0. The obtained OH form of the adsorbent was washed in the same mode with 100 ml of 1% PAA, pH 9.0 at a linear flow-rate of 60 cm/h. Finally PAA-treated Amberlite (PAA–Amberlite) was washed with deionized water in order to remove unbound polyacid.

2.3. Preparation of cross-linked PAA–Amberlite IRA-458 (cl.-PAA–Amberlite)

The cross-linking of PAA layer on the surface of Amberlite beads was carried out using a procedure including water soluble carbodiimide, EDC and using 1,6-hexamethylenediamine as a cross-linking agent. Amberlite IRA-458 was partially transformed from the Cl form into the OH form using the following procedure: adsorbent (5.5 ml) was suspended in deionized water, placed in the column (15×1 cm I.D.) and washed with 100 ml of deionized water and 20 ml of 0.1% NaOH at a linear flow-rate of 300 cm/h with all the washing procedures and pulse injections being performed in an upward mode. After the treatment with NaOH the bed was washed with 30–40 ml of deionized water. Finally, 30 ml of solution containing 2% (w/v) PAA (M_w 170 000) and 0.2% (w/v) PAA (M_w 2100) was applied to the column at a linear flow-rate of 30 cm/h. PAA-coated matrix was washed with water from unbound PAA and transferred to a beaker. The decanted suspension of modified adsorbent was resuspended in 3 ml of deionized water. The solution of carbodiimide (252 mg in 2 ml, pH 6.5–7.0) was slowly added to the gel suspension at constant stirring. The mixture was incubated for 30 min at continuous gentle stirring and adjusting pH to 6.5–

7.0 by adding 1 *M* HCl. After incubation the pH was adjusted to 8.5–9.0 by adding 1 *M* NaOH and 2 ml of an aqueous solution of 1.6-hexamethylenediamine (15 mg/ml, pH 8.5–9.0) was added. The mixture was incubated for 24 h at room temperature and 24 h at 4°C at continuous shaking. Then the gel was decanted, resuspended in 10 ml of deionized water and put on a shaker for 30 min. The washing procedure was repeated three times. Finally the gel was transferred to the column and washed with deionized water containing 0.1 *M* NaCl until absorbance of the washings at 220 nm was close to zero.

All the experiments described in the present study were carried out with the OH-form of the native, PAA– and cl.-PAA– Amberlite. The adsorbent was transformed into OH-form before each chromatographic run by washing the bed with four bed volumes of 4% NaOH and deionized water as described above.

2.4. PAA assay by turbidimetric titration using PDDPC

A calibration curve for the determination of PAA concentration by turbidimetric titration with PDDPC was obtained using the standard solutions of PAA (0.027, 0.039, 0.053, 0.079 and 0.105 mg/ml, respectively) in 20 m*M* Tris–HCl buffer, pH 7.0. The titration was carried out as follows: small aliquots (about 5 μl) of PDDPC solution (1 mg/ml) were added stepwise to the PAA solution (2 ml) at stirring. The change of turbidity after addition of each aliquot was monitored spectrophotometrically at 470 nm. New portions of PDDPC were added only after absorbance reading at 470 nm became constant. The maximum turbidity in the sample observed at a certain point of the titration corresponded to a certain PDDPC:PAA ratio, which was taken as equivalent point where the PAA negative charges were completely compensated by positive PDDPC charges. The turbidimetric titration of PAA solution of each concentration was performed three times. The confidence interval was estimated as 7%. The molar concentration of the polymers was always calculated per monomer unit of the polymer chain. The analyzed samples were thoroughly dialyzed against the control buffer before the titration.

The molar concentration of PAA was calculated taking into consideration the presence of water (15%) in the commercially available preparation of PAA.

2.5. Batch adsorption of yeast cells on (i) native and PAA-treated Amberlite IRA-401, (ii) Amberlite IRA-458 and cl.-PAA–Amberlite IRA-458

The adsorbents (0.5 ml of the adsorbent suspended in equal volume of deionized water, pH 7.0) were incubated with 2.5 ml of water containing different concentrations of yeast cells [0.8–10.0 and 0.25–5.00 mg/ml for batch adsorption on Amberlite IRA-401 (i) and Amberlite IRA-458 (ii), respectively] pH 7.0, for 1 h at room temperature and constant shaking. After incubation on a shaker the adsorbent was settled and the contents of unbound cells in the supernatants were analyzed by measuring absorbance at 620 nm. In the control experiments, 2.5 ml of water, pH 7.0, was used instead of suspensions of Amberlite.

2.6. Batch adsorption of shikimic acid on the native and cl.-PAA–Amberlite IRA-458

Non-treated or cl.-PAA-coated Amberlite (0.5 ml of the adsorbent suspended in equal volume of deionized water, pH 7.0) was incubated with 2 ml of water containing different concentrations of shikimic acid (2.4–47.0 mg/ml), pH 7.0, for 1.5 h at room temperature and constant shaking. After the incubation the gel was settled and the content of unbound shikimic acid in the supernatants was analyzed by measuring absorbance at 220 nm. In a control experiment, deionized water was used instead of Amberlite suspension.

2.7. Fluidized bed adsorption of shikimic acid and yeast cells on the native, PAA– and cl.-PAA–Amberlite

The settled bed of adsorbent (5–6 ml) in a column (15×1 cm I.D.) was transformed into a fluidized mode by pumping deionized water, pH 7.0, upwards

through the bed at a linear flow-rate of 610 cm/h. A solution of shikimic acid (10 or 20 mg/ml) or a suspension of yeast cells (10 mg/ml) was applied upwards through the bed at the same linear velocity. The content of yeast cells or shikimic acid in the effluent during adsorption, washing and elution stages was analyzed by measuring absorbance at 620 and 230 nm, respectively.

2.8. Model studies using fermentation broth from industrial fermentations

A cell-free fermentation broth from industrial fermentations of shikimic acid was provided by BioGaia and contained about 10 mg/ml shikimic. The fermentation broth was applied on a column with freshly prepared cl.-PAA–Amberlite (5 ml) in an upward mode at a linear flow-rate of 610 cm/h. The column was thoroughly washed with deionized water and the bound shikimic acid was eluted in an upward mode (flow-rate of 200 cm/h) with 3 *M* acetic acid. The resin was regenerated by washing with (i) 0.1 *M* NaCl until the disappearance of adsorbance at 220 nm in the effluent, (ii) deionized water, (iii) 20 bed volumes of 1% NaOH, (iv) deionized water till pH of the effluent was around 7.0. The regenerated column was used either for the next cycle (application of a new portion of the fermentation broth) or for analysis of the interaction of the resin with yeast cells. The analysis of the interaction of cl.-PAA–Amberlite with yeast cells was carried out after the first, the second, the fourth and the fifth cycles. It was carried out as follows: the suspension of yeast cells (30 ml) containing 10 mg/ml cells, pH 7.0–7.5 was applied to the columns in an upward mode at a linear flow-rate of 610 or 300 cm/h (at which only 1.5 times expansion was achieved). The amount of cells washed out from the column was determined spectrophotometrically by measuring absorbance at 620 nm in the effluent.

3. Results and discussion

A reliable method for the detection of PAA in the samples is required for the evaluation of the stability of the introduced PAA layer. The PAA molecule has no chromophore groups in its structure and adsorbs light only in the far UV region. In many cases spectrophotometric detection of PAA by measuring absorbance at 220 nm cannot be carried out due to the presence of other organic compounds in the analysed preparations. Thus, the development of the reliable technique for PAA detection in solutions of different composition is of critical importance for evaluation of the stability of the PAA layer at the surface of the adsorbent.

3.1. PAA assay by turbidimetric titration

A method for the detection of PAA has been developed based on the ability of polyacids to form insoluble complexes with oppositely charged polyelectrolytes. When the number of negative and positive charges in the solution of two polyions is equal the formed polycation–polyanion complex is neutral and precipitates from the solution [7]. The addition of the excess of one of the components of the complex may result in recharging of the complex and, consequently dissolution of the precipitate. The phase transitions are easy to detect by measuring the turbidity of the solution at 470 nm. The maximum of absorbance at 470 nm corresponds to the equivalence point, polyanion:polycation ratio at which the polyelectrolyte complex is neutral. In the present study the turbidimetric titration of PAA with PDDPC was used for the detection of PAA in the samples in which PAA could not be determined directly by measuring absorbance at 220 nm due to the presence of other organic compounds. The optimized procedure for the turbidimetric titration is described in Materials and methods. Fig. 1 presents the turbidimetric titration of solutions containing different concentrations of PAA (0.027–0.105 mg/ml) with the PDDPC in 20 m*M* Tris–HCl buffer, pH 7.0 (control buffer). The similar series of titrations were carried out in the control buffer containing 0.3 *M* NaCl. At a high ionic strength, the equivalence point could be detected only for relatively high PAA concentrations (0.053 and 0.105 mg/ml) but no maximum in absorbance at 470 nm was observed when titrating samples containing low (0.027 mg/ml) PAA concentration (data not shown). In the salt-free media, the equivalence point of PAA titration with PDDPC was also detected for a low concentration of PAA (0.027 mg/ml) (Fig. 1).

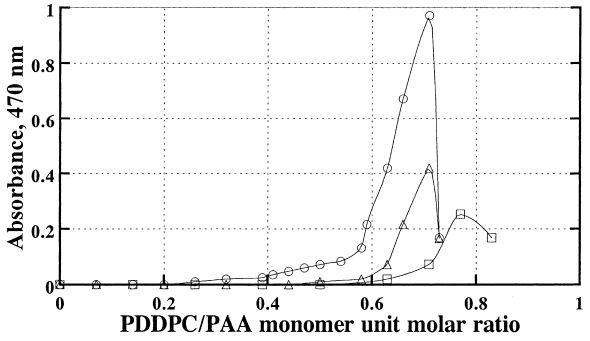

Fig. 1. Turbidimetric titration of solutions containing different concentrations of PAA with PDDPC (1 mg/ml). Experimental conditions: 20 m*M* Tris–HCl, pH 7.0. Concentration of PAA in the samples: 0.105 mg/ml (circles); 0.053 mg/ml (triangles) and 0.027 mg/ml (squares).

In order to eliminate the salt effect on turbidimetric titration, the samples of PAA were dialysed against 20 m*M* Tris–HCl buffer, pH 7.0. After dialysis the samples were analyzed by turbidimetric titration with PDDPC. The turbidimetric curves obtained for the dialyzed samples were identical to those obtained for control samples prepared in salt-free solution (data not shown). The curves for determination of concentration of PAA were plotted on the basis of the data obtained by turbidimetric titrations of PAA of different concentrations in the dialysed and control buffers (Fig. 2). The curve for the dialysed samples lies slightly lower than the blank. Probably, this was due to some leakage of low-molecular-weight polymer fraction present in the commercial PAA preparation through the dialysis membrane (M_r cut-off 12 000–14 000).

3.2. Adsorption of shikimic acid and yeast cells by PAA–Amberlite IRA-401

Yeast cells, which were used in this study as model microorganism, are negatively charged at neutral conditions. Hence they have a strong tendency to adsorb to positively charged matrices like Amberlite IRA-401. (Fig. 3, open symbols). However, practically no cell binding was observed under the same conditions during incubation of cells with PAA-treated Amberlite, i.e. not more than 3–6% of applied biomass was absorbed to the modified adsorbent (Fig. 3, closed symbols). This was probably due to the cell repulsion by the negatively charged polymer chains.

The fluidized mode of adsorption has been used to enable application of cell-containing suspension on an ion-exchange column. Cell-containing suspensions cannot be applied on a packed bed column as the bed presents a depth filter, which retains cells. The cell retention by a packed-bed column results in complete blockage of the flow through the column.

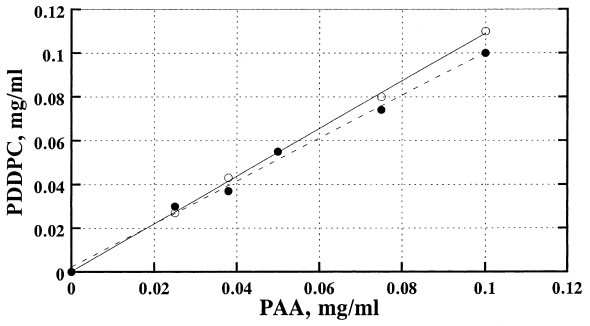

Fig. 2. Determination of the concentration of PAA by turbidimetric titration with PDDPC. Experimental conditions: 20 m*M* Tris–HCl buffer, pH 7.0 (control buffer, open circles) and 20 m*M* Tris–HCl buffer, pH 7.0, initially containing 0.3 *M* NaCl and dialyzed against the control buffer (closed circles). Concentration of added PDDPC was 1 mg/ml.

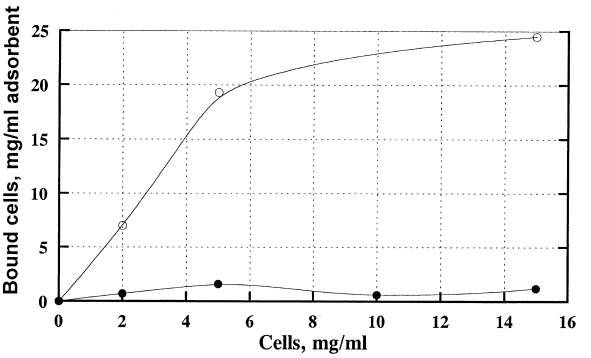

Fig. 3. Adsorption of yeast cells to the native (open circles) and PAA- (closed circles) Amberlite IRA-401. Experimental conditions: 0.5 ml of the adsorbent suspended in equal volume of deionized water was incubated with 2.5 ml of water containing different concentrations of yeast cells for 1 h at pH 7.0, room temperature and constant shaking. Cell content was detected by optical density at 620 nm.

The fluidization of the Amberlite was achieved at a linear flow-rate of 610 cm/h with an 1.8-fold bed expansion. The beds of both native and PAA-coated Amberlite had some back mixing of the adsorbent and represented a mixed mode between completely fluidized bed and expanded bed.

A pulse of cells (10 mg/ml, 30 ml) was passed through fluidized beds of the native and PAA-treated Amberlite IRA-401 in an upward mode. As shown in Fig. 4a, up to 26% of applied cells were retained by the native matrix and approximately 13% by the PAA-treated matrix (Fig. 4b). The biomass strongly bound to the native Amberlite and practically was not washed out from the bed during the washing stage (Fig. 4a). Bound cells caused aggregation of native beads and formation of channels in the fluidized bed was visually observed. However, approximately 98% of retained cells were washed out from PAA–Amberlite IRA-401 during the washing stage (Fig. 4b) and no aggregates were observed in this case. Thus, coating of the strong anion-exchange matrix with a layer of PAA proved to be effective against undesirable interaction with the cells.

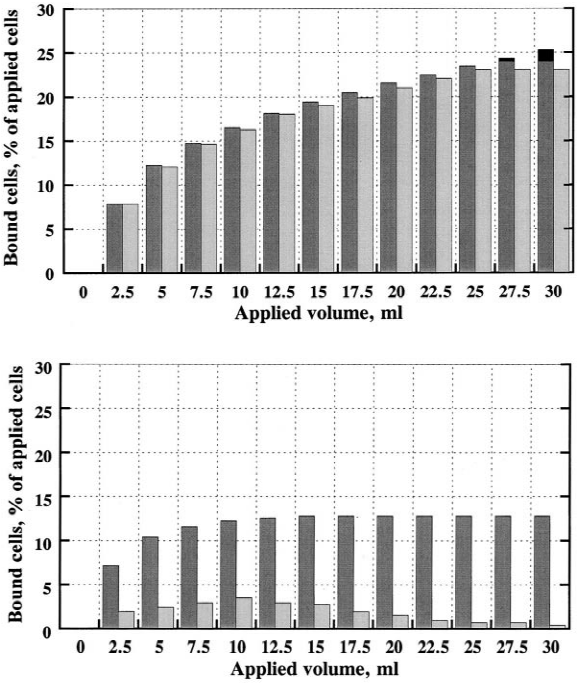

Fig. 4. Amount of yeast cells bound to the native (a) and PAA- (b) Amberlite IRA-401 in a fluidized bed adsorption before (dark grey columns) and after (light grey columns) washing the bed of adsorbent with deionized water. Experimental conditions: 5 ml settled bed volume in a 15.0×1 cm I.D. column at room temperature. The suspension of yeast cells (30 ml; 1%, w/v), pH 7.0, was passed through the column equilibrated with deionized water in an upward mode at a linear velocity of 610 cm/h.

Adsorption of PAA to the anion-exchanger results in the occupation of some ion-exchange ligands and hence in a reduced capacity of the matrix towards a low-molecular-mass target substance, e.g. shikimic acid. To evaluate the significance of the capacity decrease caused by PAA adsorption, the breakthrough curves for shikimic acid on native and PAA–Amberlite IRA-401 were compared (Fig. 5). The close similarity of the breakthrough profiles for shikimic acid on both adsorbents indicate that the shielding PAA layer was bound mainly at the surface of the beads without affecting much the dynamic binding capacity of the matrix. Probably, the chains of PAA with high molecular mass did not diffuse inside the pores of the beads and thus did not decrease much the total capacity of the adsorbent while effectively shielding the matrix from the interactions with yeast cells.

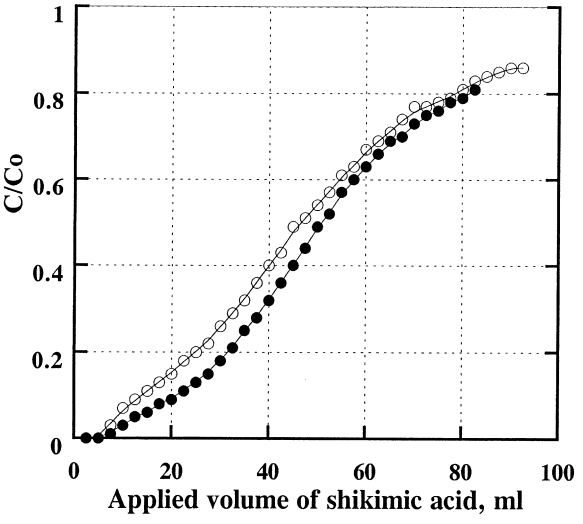

Fig. 5. Breakthrough of shikimic acid on the native (open circles) and PAA– (closed circles) Amberlite IRA-401. Experimental conditions: 5 ml settled bed volume in a 15×1 cm I.D. column at room temperature. Shikimic acid (0.5%, w/v) pH 7.0, was passed through the column equilibrated with deionized water at a linear velocity 610 cm/h.

3.3. Cross-linking of PAA layer adsorbed to Amberite IRA-458

High concentrations of acids used for eluting low-molecular-mass fermentation products adsorbed to Amberlite matrices could be detrimental for the stability of a shielding PAA layer. In fact, strong leakage of PAA was observed when 3 *M* acetic acid was used to elute bound shikimic acid. Fresh Amberlite IRA-458 adsorbed about 6.2 mg PAA per ml adsorbent (settled volume). Elution of bound shikimic acid with eight bed volumes of 3 *M* acetic acid and careful analysis of the eluted fractions for PAA showed that about 70% of adsorbed PAA was stripped from the matrix. The second cycle of the elution with 3 *M* acetic acid resulted in nearly quantitative removal of PAA from the PAA–Amberlite IRA-458 (Table 1). Thus, covalent fixation of the PAA layer is needed to ensure its stability under conditions of the binding–elution cycle.

A chemical cross-linking of the PAA layer was carried out according to the following scheme: (i) PAA binding to the matrix via electrostatic interactions, (ii) activation of PAA carboxy groups with water-soluble carbodiimide, (iii) cross-linking of activated PAA molecules with 1,6-hexamethylenediamine. The procedure of binding of the polymer to the resin was modified as compared to the standard procedure described above. This modification was introduced in order to obtain the Cl form of PAA–Amberlite, while the standard procedure resulted in the formation of OH form of PAA-coated adsorbent. The appropriate adjustments of pH required for the cross-linking reaction were not possible in the case of PAA–Amberlite in OH form. The addition of carbodiimide solution (pH 5.0) to the suspension of the adsorbent in the OH form with pH 7.0–8.0 resulted in a dramatic increase in pH to 11.0–12.0. Probably, this was due to the binding of Cl^- (counterions for positively charged diethylaminoopropyl group in EDC) and release of equivalent amount of OH^- from the Amberlite. To overcome the problem, Amberlite IRA-458 was only transformed partially from the Cl form into the OH form prior to PAA treatment. This was achieved by decreasing the amount of NaOH used for the replacement of Cl^- ions on the adsorbent with OH^- ions. When using only four bed volumes of 0.1% NaOH (instead of 4%) the matrix acquire sufficient amount of OH form to ensure binding PAA, while the major part remains in the Cl form. A small amount (0.2% w/v) of PAA with low molecular mass (2 100) was added to the 2% (w/v) solution of PAA with high molecular mass (170 000) used for coating the matrix. The fraction of 'small' polyacid was added in order to neutralize OH groups which could be inside the pores of the beads and thus were unavailable for the large PAA molecules. The limited number of OH groups on the resin was still sufficient for binding PAA (30.5 mg/ml adsorbent). The layer of PAA adsorbed in this way was successfully cross-linked using the carbodiimide–1,6-hexamethylenediamine procedure as described in Materials and methods giving cl.-PAA–Amberlite.

Only minute amount of bound PAA (0.2 and 0.1% in cycle I and II, respectively) leaked from cl.-PAA–Amberlite when eluting shikimic acid with 3 *M* acetic acid.

Table 1
Binding capacity of the cl.-PAA– and native Amberlite. The fermentation broth was applied on a column with 5 ml adsorbent (settled volume) in an upward mode at a linear flow-rate of 610 cm/h. The column was thoroughly washed with deionized water and the bound shikimic acid was eluted in an upward mode (flow-rate of 200 cm/h) with 3 *M* acetic acid

Cycle	Binding capacity of cl.-PAA–Amberlite, shikimic acid (g/l)	Binding capacity of native Amberlite, shikimic acid (g/l)
I	16.8	14.7
II	16.3	No bed expansion — further use of the column impossible
III	15.9	
IV	14.7	
V	14.0	

3.4. Properties of cl.-PAA–Amberlite

The modified adsorbent, cl.-PAA–Amberlite displayed improved flow properties as compared to the unmodified resin. Fig. 6 presents the dependence of the bed height of cl.-PAA and native Amberlite on the flow-rate of mobile phase. Both curves were obtained after the gels were pretreated by washing with four bed volumes of 3 *M* acetic acid followed by washing with deionized water. The pretreatment

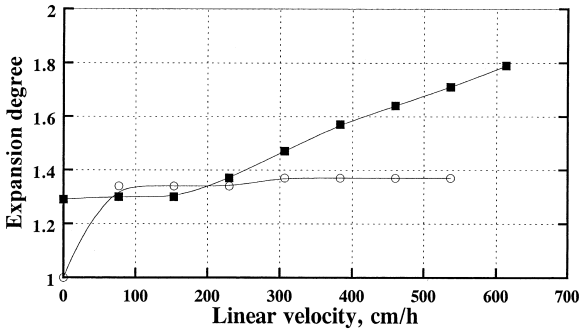

Fig. 6. Dependence of the expansion degree of the bed of the native (open circles) and cross-linked PAA- (closed squares) Amberlite IRA-458 on the linear fluid velocity. Experimental conditions: 5 ml settled bed volume in a 15×1 cm I.D. column at room temperature. The bed of adsorbent was fluidized with deionized water applied in an upward mode at various linear velocities.

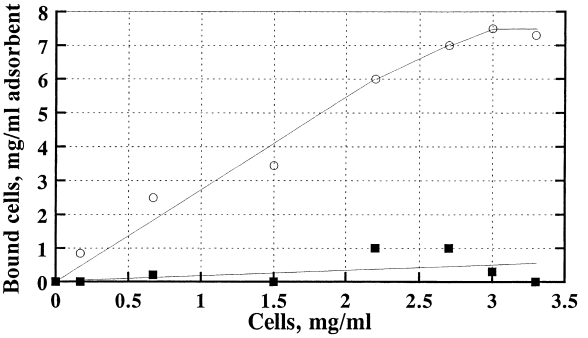

Fig. 7. Adsorption of yeast cells to the native (open circles) and cross-linked PAA– (closed squares) Amberlite IRA-458. Experimental conditions: 0.5 ml of the adsorbent suspended in equal volume of deionized water was incubated with 2.5 ml of water containing different concentrations of yeast cells for 1 h at pH 7.0, room temperature and constant shaking. Cell content was detected by optical density at 620 nm.

of the native Amberlite IRA-458 with 3 *M* acetic acid resulted in the changes in its flow properties. The bed of the native gel pretreated with acetic acid did not expand much under applied flow as the particles of the resin seemed to be 'glued' together. The bed was not fluidized even at flow-rates as high as 530 cm/h. The cl.-PAA–Amberlite was, however, easily fluidized. The degree of expansion of the bed increased with the increase in the flow-rate (Fig. 6). The degree of expansion of 1.8 was achieved at a linear flow-rate of 610 cm/h.

The binding of yeast cells to cl.-PAA–Amberlite in a batch mode was considerably reduced as compared to the binding to the native matrix (Fig. 7). On the other hand, batch adsorption experiments demonstrated that the introduction of a cross-linked PAA layer only slightly influenced the capacity of the ion exchanger for the target product, shikimic acid (Fig. 8). The similar results were obtained in a fluidized mode: 81.3 and 80.6 mg of shikimic acid bound per ml adsorbent to the native and cl.-PAA–Amberlite, respectively.

The PAA content in shikimic acid preparations eluted from the modified matrix with 3 *M* acetic acid was below detection limit already after the second elution cycle. Yeast cells applied on a column with a fluidized cl.-PAA–Amberlite bed did not bind to the matrix and were completely washed out with deionized water. The matrix was successfully used in three cycles of binding–elution of the shikimic acid in a model system comprised of shikimic acid and yeast cells.

A more realistic model of the process was the filtered fermentation broth from the industrial fermentation of shikimic acid with yeasts added as model cells. The capacity of cl.-PAA–Amberlite towards shikimic acid decreased slightly with the increasing numbers of application cycles for the cl.-PAA–Amberlite. Nevertheless the drop in capacity after five cycles did not reach above 20%. The

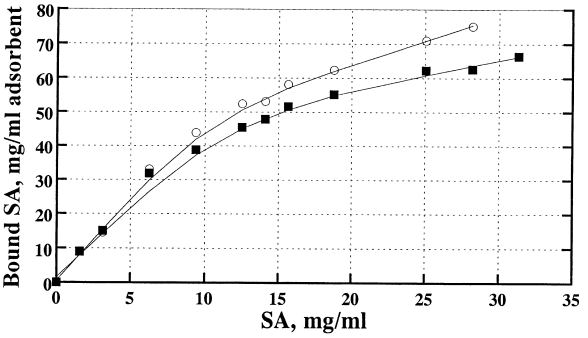

Fig. 8. Adsorption of shikimic acid (SA) to the native (open circles) and cross-linked PAA- (closed squares) Amberlite IRA-458. Experimental conditions: 0.5 ml adsorbent suspended in equal volume of deionized water was incubated with 2 ml of water containing different concentrations of shikimic acid for 1.5 h at pH 7.0, room temperature and constant shaking.

Table 2
Interaction of the yeast cells with cl.-PAA- and native Amberlite. The suspension of yeast cells (30 ml) containing 10 mg/ml cells, pH 7.0–7.5 was applied to the columns in an upward mode at a linear flow-rate of 610 or 300 cm/h. The amount of cells washed out from the column was determined spectrophotometrically by measuring absorbance at 620 nm in the effluent

Type of carrier	Linear velocity (cm/h)	Amount of bound cells (mg/ml)
cl.-PAA–Amberlite	610	0.0
cl.-PAA–Amberlite	300	1.4
Native Amberlite	610	3.6

application of yeast cell suspension did not result in any visible decrease of the cl.-PAA–Amberlite performance and no binding of cells to the cl.-PAA–Amberlite has been detected. Some yeast cells were entrapped in the cl.-PAA–Amberlite bed at lower flow-rate of 300 cm/h (1.5 fold bed expansion) (Table 2), but those cells were easily removed by washing at the flow-rate of 610 cm/h (1.8-fold bed expansion) used throughout the most of experiments.

However, the application of yeast cells in the column with native Amberlite resulted in the strong binding of about 3 mg of cells per ml of the adsorbent. The performance of the native Amberlite with bound cells was deteriorated even after a rigorous washing procedure including washing with 20 bed volumes of 1% NaOH. Already, after the first cycle, the bed of native Amberlite did not expand any more at the flow-rate 610 cm/h and the further use of the adsorbent was impossible (Table 1).

Finally, cl.-PAA–Amberlite was used for the direct capture of shikimic acid from the cell-containing fermentation broth. The binding capacity of the adsorbent was 10.1 mg of shikimic acid per ml of adsorbent and thus, was lower than the capacity observed in the experiments with the cell-free fermentation broth. The decrease in the capacity could be due to the binding of some other components present in the fermentation broth. However, no clogging of the column was observed and the performance of cl.-PAA–Amberlite did not deteriorate after the application of the broth. Practically the same amount of the target product (9.7 mg/ml adsorbent) was bound in the second chromatographic cycle when using the whole fermentation broth.

4. Conclusion

The results presented in this study clearly indicate that the adsorption of the negatively charged polymer, PAA, on strong cation exchanger with high capacity like Amberlite significantly reduces undesirable adsorption of cells without significant loss of binding capacity for the target product. The PAA-treated Amberlites could be used for the direct capture of fermentation products, like shikimic acid, directly from the cell-containing fermentation broth using fluidized bed mode of adsorption.

The stability of the shielding layer of PAA under harsh elution conditions was greatly improved by its chemical modification via cross-linking. The Amberlite with cross-linked PAA layer displayed the same favourable characteristics: good capacity for the target product and resistance to cell binding. Thus, the coating of adsorbents with polyelectrolytes has a high potential for the direct capture of low-molecular-mass products from fermentation broths.

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