# Pomegranate Peel as Bio-adsorbent for Nutrients Removal and Recovery from Aqueous Solutions

**Thesis booklet**

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## 1 Introduction and background of research

Nutrients removal has become one of the key challenges for wastewater treatment facilities due to the harmful impact of these pollutants including ammonium ($NH_4^+$) and phosphate ($PO_4^{3-}$) on human health, water bodies and ecosystems. Available methods for removal of these contaminants come with several disadvantages mainly investment and operational costs that are hard to handle in regions with lack of appropriate budgets for wastewater treatment systems. However, the use of Agricultural and Food Waste (AFW) as bio-adsorbents for nutrients removal and recovery from wastewater presents an eco-friendly, innovative and sustainable approach for water, nutrients and solid waste management according to the 4R principle (Reduce, Recycle, Reuse and Recover); Reduce the overuse of water and fertilizers; Recycle wastewater, Reuse AFW and Recover nutrients. AFW, also called ligno-cellulosic biomass such as stalks, leaves, seeds, shell, peels, husks, and straws are composed of hemicelluloses, lignin, lipids, proteins, simple sugars, water, hydrocarbons and starch, containing a variety of functional groups especially hydroxyl (–OH) and carboxyl (–COOH) groups that afford them a promising ion-exchange capacity and general adsorptive characteristics. Several AFW have been studied for $NH_4^+$ removal from water [1,2]. Although, the adsorption capacity vary depending on the type of AFW, most of these biomaterials can be used in natural form for $NH_4^+$ removal, however, their activation is highly required in order to develop desirable physicochemical properties for $PO_4^{3-}$ removal [3]. However, the use of AFW as bio-adsorbents for nutrients removal and recovery should take in consideration several criteria such as abundancy, cost effectiveness, high efficiency at a short time, ease of regeneration after use, and the possibility of reuse.

Among the widely abundant AFW, pomegranate peel (PP) occupies a leading position; approximately 1.5 million tons of PP is produced per year [4]. Consequently, PP present a major environmental concern for producer countries (e.g., India, Iran, Turkey, China, United States of America, Egypt, Spain, Italy, Tunisia, Morocco, Argentina, Brazil, Australia and others) [5]. Nowadays, there is a trend toward using PP as bio-adsorbent for heavy metals, dyes and other contaminants removal due to several advantages that present this management method [6–9]. However, it is believed that the potential application of PP as bio-adsorbent has not been fully explored. Therefore, this Ph.D. thesis investigated for the first time the use of PP for $NH_4^+$ and $PO_4^{3-}$ removal and recovery.

## 2 Applied Methods

### 2.1 Bio-adsorbent preparation and screening experiment

Seven AFW were collected, including pomegranate peel, banana peel, wheat husk, compost, poplar bark, wheat bran and sugar beet pulp to test their efficiency to remove $NH_4^+$ from ammonium chloride model solution ($NH_4Cl$) using batch adsorption method. For this purpose samples were cut into small pieces and washed with distilled water several times to remove dust and impurities, then they were oven dried at 110 °C for two hours. Finally, dried materials were crushed and grinded to desired particle size (< 250 μm). Then, 100 mg of each sample was introduced in 60 mL of $NH_4Cl$ solution with an initial $NH_4$-N concentration of 30 mg.L$^{-1}$. The system was shaken at 100 rpm and at room temperature until equilibrium. For the determination of $NH_4^+$ removal, residual solutions were filtered using 0.45 μm microporous membrane filters. $NH_4$-N concentration was determined by spectrophotometer Spectroquant Nova 60 (Merck, Germany), using test kits that are automatically identified and the measurement value can be immediately read off from the display. $NH_4^+$ removal rate can be calculated as shown in Eq. (1).

$$\text{Removal}\,\% = \frac{C_i - C_f}{C_i} * 100 \qquad (1)$$

Where: $C_i$ and $C_f$ are the initial and final $NH_4$-N concentrations in solution, respectively.

### 2.2 Activation of PP

The activation of PP was done using an approach similar to that of Nguyen and coworkers based on iron loading method in order to improve $PO_4^{3-}$ removal ability [10]. The first step of this activation method was the base treatment or saponification, where 40 g of PP was stirred with 1 L of sodium hydroxide solution (NaOH, 0.05 M) at room temperature for 24 h and then washed several times with distilled water until the pH of washing solution became neutral. The second step was the iron loading, where the saponified PP was stirred with 500 mL of iron chloride solution ($FeCl_3$, 0.25 M) at room temperature for 24 h. Finally, the iron-loaded PP (IL-PP) was carefully washed with distilled water again and oven-dried at 105°C for 8 h, and then, it was mechanically milled with a planetary ball mill to the desired size (<250 μm) before use in the adsorption of $PO_4^{3-}$ from di-sodium hydrogen phosphate model solution ($Na_2HPO_4$).

## 2.3 Characterization of PP and IL-PP

- **Particle size distribution and porosity :**

The particle size distribution of PP was determined using laser particle size analyzer in dry mode Horiba LA-950V2 (Horiba, Japan). From the measured data, the computer calculated the particle size distribution according to the Fraunhofer theory. Measurement range of the analyzer is between 10 nm and 3 mm. While, the porosity of PP was determined using automated mercury porosimeter AutoPore IV 9500 Series (Micromeritics, USA). This device characterizes material's porosity by applying various levels of pressure to a sample immersed in mercury then the pressure readings are converted to pore diameter by means of the Washburn equation or by another model.

- **Zeta potential**

Zeta potential of PP and IL-PP were determined using 10 mg suspensions mixed in bottles containing 30 ml of $NH_4Cl$, sodium chloride (NaCl) and $Na_2HPO_4$ solutions at different concentrations and pH values. After mixing, equilibrium pH of samples was measured and adjusted then zeta potential was measured by zetasizer Nano Zs, (Malvern, UK) using electrophoretic light scattering (ELS). All samples were prepared in triplicates and the average of measurements was used for data analysis.

- **FT-IR ATR analysis**

PP and IL-PP surfaces were investigated using FTIR-ATR analysis. The instrument for recording the spectra was spectrophotometer Bio-Rad Digilab Division FTS-65A/896 (Bio-Rad, USA) with 4cm$^{-1}$ resolution. The 4000–400 cm$^{-1}$ wavenumber range was recorded and 256 scans were collected for each spectrum. In addition to the spectra of each sample, single reflection of diamond ATR accessory measurements were taken.

- **Scanning electron microscope SEM**

SEM was used for imaging the microstructure of PP and IL-PP using Hitachi S-4700 type II scanning electron microscope (Hitachi, Japan). For the production and acceleration of the electron beam, a cold field emission gun and 10 kV acceleration voltage were applied, respectively. The micrographs were recorded by collecting secondary electrons with an Everhart–Thornley detector.

### 2.4 Milking parlour wastewater (MPWW)

Wastewater used for $NH_4^+$ biosorption study was sampled from a milking parlour unit near the city of Szeged, Hungary on 13/04/2019. 20 L volume of MPWW was generated and stored in the laboratory's freezer before characterization and use for the adsorption experiments. Chemical oxygen demand (COD) was measured using the colorimetric method (APHA 5220D 2005). 5 days biochemical oxygen demand ($BOD_5$) was determined by respirometric method using ovibond Oxidirect (Germany) at a controlled temperature of 20 °C for a 5-day-long period. Total nitrogen (TN) content was determined by Torch (Teledyne Tekmar, U.S.) combustion (HTC) type analyzer equipped with pressurized NDIR detector. Ammonium content was determined using spectrophotometry method by Spectroquant Nova 60 (merck, Germany).

### 2.5 Batch adsorption studies

- **Adsorption of $NH_4^+$ from $NH_4CL$ and MPWW**

Batch adsorption method was used in order to study the equilibrium characteristics of $NH_4^+$ adsorption by PP from $NH_4Cl$ solution and MPWW. The first series of experiments were carried out to assess the effect of parameters such as initial $NH_4$-N concentration, pH, adsorbent dose, stirring speed, temperature and contact time using the one factor at a time method (OFAT). For the measurement of $NH_4$-N concentration, method used in the screening experiments was followed and $NH_4^+$ removal rate was calculated using Eq. (1).

For isotherm and kinetics modelling, data were fitted to the existing models using linear method and the best-fit models were selected based on the highest correlation coefficient ($R^2$) and agreement of experimental data with calculated parameters.

The adsorbed amount of $NH_4$-N was calculated using Eq. (2).

$$q_e = (C_i - C_e)\frac{V}{m} \qquad (2)$$

where: $q_e$ (mg.g$^{-1}$) is the amount of $NH_4$-N per mass unit of adsorbent at equilibrium, $C_i$ (mg.L$^{-1}$) and $C_e$ (mg.L$^{-1}$) are the initial and equilibrium $NH_4$-N concentrations in solution, respectively. *V* (*L*) is the solution volume and *m* (g) is the mass of PP.

- **Adsorption of $PO_4^{3-}$ from $Na_2HPO_4$ model solution**

Similar method and equations described for $NH_4^+$ adsorption study were used to determine $PO_4^{3-}$ removal and $PO_4$-P adsorbed amount, however, to identify the influencing and optimum parameters for higher $PO_4^{3-}$ removal, factorial design using Minitab19 software was applied instead of OFAT method.

In order to identify isotherm and kinetic models that adequately describe $PO_4^{3-}$ adsorption by IL-PP, non-linear method was performed using the Solver add-in, Microsoft Excel, which is based on values of non-linear correlation coefficients ($R^2$) and chi-square ($\chi^2$) that can be calculated using Eq. (3) and Eq. (4), respectively:

$$\chi^2 = \sum \frac{(q_{e,exp} - q_{e,cal})^2}{q_{e,cal}} \qquad (3)$$

$$R^2 = \frac{\sum(q_{e,cal} - q_{e,mean})^2}{\sum(q_{e,cal} - q_{e,mean})^2 + \sum(q_{e,cal} - q_{e,exp})^2} \quad (4)$$

Where: $q_{e,exp}$ ($mg.g^{-1}$) is the amount of $PO_4$-P uptake at equilibrium, $q_{e,cal}$ ($mg.g^{-1}$) is the amount of $PO_4$-P uptake achieved from the model using the Solver add-in, and $q_{e,mean}$ ($mg.g^{-1}$) is the mean of the $q_{e,exp}$ values.

For describing thermodynamic properties of $PO_4^{3-}$ adsorption by IL-PP, thermodynamic parameters including ΔG°, ΔH° and standard ΔS° were calculated using Eq. (5) and Eq. (6).

$$\Delta G^0 = -R\,T\,lnK_d \quad (5)$$

$$lnK_d = \frac{\Delta H^0}{R\,T} + \frac{\Delta S^0}{R} \quad (6)$$

where: T is the absolute temperature in kelvin, R is the gas constant (8.314 $J.mol^{-1}.K^{-1}$) and $K_d$ presents the distribution coefficient.

## 2.6 Application of nitrogen-loaded PP (N-PP) and phosphorus-loaded IL-PP (P-IL-PP) as fertilizer

- **Germination test**

The phytotoxicity effect of Raw-PP, N-PP and P-IL-PP was studied using rapeseed (Brassica napus L.). For this purpose, ten seeds of Brassica napus L. were placed and equally distributed on filter paper in 9-cm diameter Petri dishes. Raw-PP, N-PP and P-IL-PP were suspended in 20 mL of distilled water for use as a medium for seeds germination, and then 3 mL of each of the following treatments was added in triplicate:

- ✓ Distilled water for the control
- ✓ Raw-PP (0.05 %, 0.5% and 5% of dry matter)
- ✓ N-PP (0.05 %, 0.5% and 5% of dry matter)
- ✓ P-IL-PP (0.05 %, 0.5% and 5% of dry matter)

- ✓ Mixture of N-PP and P-IL-PP (0.05 %, 0.5% and 5% of dry matter)

The petri dishes were incubated at 25°C in a dark incubator for 4 days. Seeds with visible roots after 4 days were counted as germinated. Root lengths were measured from the transition points among the hypocotyl to their extremities based on their digital images using ImageJ software. Then, relative seed germination (RSG), relative root elongation (RRE), and germination index (GI) were calculated using the following equations.

$$\mathrm{RSG}(\%) = \frac{\text{Number of seeds germinated in the sample extract}}{\text{Number of seeds germinated in the control}} \times 100 \qquad (7)$$

$$\mathrm{RRE}(\%) = \frac{\text{Mean root elongation in the sample extract}}{\text{Mean root elongation in the control}} \times 100 \qquad (8)$$

$$\mathrm{GI}(\%) = \frac{(\text{\% Seed germination})\times(\text{\% Root elongation})}{100} \qquad (9)$$

- **Pot experiments**

An outdoor pot experiment was conducted to examine the effects of N-PP and P-IL-PP on both vegetative and reproductive growth Brassica napus L. For this purpose, treatments used in the germination test were used for this experiment as well. 15 treatments with 3 replicates were included so that a total of 45 pots were cultivated. The treatments were manually mixed into Mr. Garden flower soil (pH: 5.5, N: 0.1% (m/m), $P_2O_5$: 0.01% (m/m), $K_2O$: 0.03% (m/m), organic matter: 75% (m/m) and 70% initial water content) using a wooden stick. Then, a 2 L polypropylene pot (15 cm diameter) was filled with 600 g of the prepared soil medium. Brassica napus L. seeds were pre-germinated for 24 h at 26 °C. Then, the germinated seeds were transplanted uniformly in pots (7 seeds each). Pots were then placed in a bright area protected from direct sunlight under natural weather conditions. Plants were watered only if necessary. After 32 days, plant habits of rapeseed seedlings were recorded and harvested and then the basic morphological parameters were measured including the number of leafs in addition to fresh and dry weights of roots and shoots.

## 3 Novel scientific results

### 3.1 Biosorption of $NH_4^+$ from $NH_4Cl$ solution using PP

- The adsorbent dose and initial $NH_4$-N concentration were the parameters with the highest impact on the process. 97% $NH_4^+$ removal was achieved within 120 min using 400 mg PP and an initial $NH_4$-N concentration of 30 mg.$L^{-1}$ at pH 4 and 100 rpm stirring rate.
- Isotherm modelling showed that the adsorption of $NH_4^+$ from $NH_4Cl$ model solution using PP is well described by the Langmuir model ($R^2$ = 0.98) with a maximum adsorption capacity of 6.18 mg $NH_4$-N/g. This model assumes that the adsorption process is localized and controlled by monolayer coverage of PP's surface and all adsorption sites possess equal affinity for $NH_4^+$. Furthermore, intermolecular attractive forces diminish rapidly with distance in absence of interaction between adsorbed molecules on neighbouring sites. Value of the Langmuir constant $k_L$ (=3.01) showed that PP has a high affinity towards $NH_4^+$. While, value of the separation factor $R_L$ (=0.01) indicated that the process is favourable.
- The process kinetics showed that the system reached equilibrium within 120 min and the process can be adequately described by pseudo-second order model ($R^2$ = 0.99). This model assumes that the process is governed by chemisorption mechanism with sharing and exchange of electrons between PP's surface and $NH_4^+$.

**Publication:** Naoufal Bellahsen, Gábor Varga, Nóra Halyag, Szabolcs Kertész, Etelka Tombácz and Cecilia Hodúr (2020): Pomegranate peel as a new low-cost adsorbent for ammonium removal. **In**: International Journal of Environmental Science and Technology **(Q2**, **IF: 2.860)**.

### 3.2 Biosorption of $NH_4^+$ from MPWW using PP

- The adsorbent dose was the parameter with the highest impact on the process. More than 81% $NH_4^+$ removal was achieved within 120 min using 1.5 g PP, an initial NH4-N concentration of 80 $mg.L^{-1}$, and 300 rpm stirring rate at pH 6 and 25 °C temperature.
- Langmuir isotherm offered the best fit to experimental adsorption curve according to the value of $R^2$ (>0.99) with a maximum adsorption capacity of 2.49 $mg.g^{-1}$, which is lower than the value obtained in $NH_4Cl$ model solution due to the competition from other cations present in MPWW. Value of $R_L$ obtained (= 0.02) was very low and close to the value obtained for adsorption of $NH_4^+$ from $NH_4Cl$ model solution (0.01), which indicated that $NH_4^+$ biosorption from MPWW by PP remain favourable. However, competition from other cations present in MPWW have decreased the value of $k_L$ from 3.01 for $NH_4Cl$ model solution to 0.42 for MPWW, which indicates a decrease in the affinity of PP towards $NH_4^+$.
  - Similarly to kinetics of $NH_4^+$ adsorption by PP from $NH_4Cl$ model solution, pseudo-second order kinetic model fits well with the adsorption of $NH_4^+$ from MPWW by PP according to the value of $R^2$ (>0.99). These results approve the governance of chemisorption mechanism on the process.

**Publication:** Cecilia Hodúr, Naoufal Bellahsen, Edit Mikó, Virág Nagypál, Zita Šereš and Szabolcs Kertész (2020): The Adsorption of Ammonium Nitrogen from Milking Parlor Wastewater Using Pomegranate Peel Powder for Sustainable Water, Resources, and Waste Management. **In**: Sustainability **(Q2, IF: 3.251)**

### 3.3 Biosorption of $PO_4^{3-}$ from $Na_2HPO_4$ solution using IL-PP

- PP was successfully modified by iron loading method for efficient adsorption of $PO_4^{3-}$ : 90% removal was achieved within 60 min using 150 mg IL-PP and an initial $PO_4$-P concentration of 40 mg.L$^{-1}$ at pH 6 and 25 °C temperature. Adsorbent dose and pH were the parameters with the highest effect on the process. Model for $PO_4^{3-}$ removal from $Na_2HPO_4$ solution using IL-PP can be written as follow:

$$\text{Removal\%} = 71.19 + 6.33\, pH + 16.28\, Adsorbent\ dose - 3.43\, pH \cdot Adsorbent\ dose + 0.06\, Ct\, Pt$$

- Biosorption of $PO_4^{3-}$ by IL-PP can be described by both Langmuir ($R^2$ = 0.98, $\chi^2$ = 0.78) and Freundlich ($R^2$ = 0.94, $\chi^2$ = 2.62) isotherms, but the former fits better than the latter with a maximum $PO_4$-P uptake of 49.12 mg·g$^{-1}$. The applicability of both Langmuir and Freundlich models indicates the homogeneous and heterogeneous distribution of active sites on IL-PP's surface and consequently the adsorption properties are likely to be complex and involve more than one mechanism. Langmuir separation factor ($R_L$= 0.21) is between 0 and 1 and the Freundlich adsorption affinity constant (n = 2.04) is between 1 and 10 which implied a favourable biosorption.
- Kinetics of $PO_4^{3-}$ biosorption by IL-PP can be best described by Elovich model ($R^2$ = 0.97, $\chi^2$ = 0.007, $q_{e,cal}$ = 12.11). This kinetic

model describes chemical adsorption mechanism and suggests that IL-PP's surface is heterogeneous.

- Standard free energy change ΔG° was found to decrease when temperature increased indicating the spontaneous nature of the process at high temperature. Positive value standard enthalpy change ΔH° indicates that $PO_4^{3-}$ biosorption by IL-PP is endothermic in nature, while positive entropy change ΔS° indicates that IL-PP has good affinity to $PO_4^{3-}$.

**Publication:** Naoufal Bellahsen, Balázs Kakuk, Sándor Beszédes, Zoltán Bagi, Nóra Halyag, Tamás Gyulavári, Szabolcs Kertészs, Ahmed El Amarti, Etelka Tombácz and Cecilia Hodúr (2021): Iron-loaded pomegranate peel as a bio-adsorbent for phosphate removal. **Under review** In: Water **(Q2**, **IF: 3.103)**

### 3.4 Application of N-PP and P-IL-PP as fertilizer

- Germination index GI of all used N-PP, IL-P-PP, and Mix treatments was above 50% indicating no inhibitory effect during the germination phase. Moreover, 0.05 and 0.5 % (N-PP, IL-P-PP, and Mix) showed a beneficial effect as their GI was higher than the control.
- Positive effect on the number of leaves produced was observed for all treatments except 0.5% P-IL-PP.
- A positive effect was observed on shoots dry and fresh weight for most of the treatments except 0.05% N-PP and 0.05% Mix.

**Publication:** In the writing phase

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## List of publications

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- Bellahsen N, Kertész S, Pásztory Z, Hodúr C (2018) Adsorption of nutrients using low-cost adsorbents from agricultural waste and by-products – Review. Prog Agric Eng Sci 14(1):1–30. https://doi.org/10.1556/446.14.2018.1.1 (Q3)
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- Hodúr C, Bellahsen N, Mikó E, Nagypál V, Šereš Z, Kertész S (2020) The adsorption of ammonium nitrogen from milking parlor wastewater using pomegranate peel powder for sustainable water, resources, and waste management. Sustain 12(12). https://doi.org/10.3390/SU12124880 (Q2, IF: 3.251)
- Bellahsen N, Kakuk B, Beszédes S, Bagi Z, Halyag N, Gyulvári T, Kertész S, El Amarti A, Tombácz E, Hodúr C (2021) Iron-loaded pomegranate peel powder as a new bio-adsorbent for phosphate removal. Water - under review (Q2, IF: 3.103).

## Presentations in conferences

- Adsorption and recovery of phosphate ions from aqueous solution using Iron-loaded pomegranate peel powder: Miskolc IPW–IV. Sustainable Raw Materials International Project Week, Miskolc, Hungary, November 25-27, 2020.

- Adsorption and recovery of phosphate ions from aqueous solution using activated pomegranate peel powder: Student Conference on Conservation Science (SCCS Europe), Tihany, Hungary, August 25-29, 2020.
- Removal and recovery of ammonium from milking parlour wastewater using pomegranate peel: 1st International Conference on Advanced Production and Processing (ICAPP), Novi Sad, Serbia, October 10-11, 2019.
- Adsorption of ammonium from milking parlour wastewater by using pomegranate peel: PERMEA 2019 - Membrane Conference of Visegrád Countries, Budapest, Hungary, August 26-29, 2019.
- Removal of ammonium from milking parlour wastewater by using pomegranate peel: II. SUSTAINABLE RAW MATERIALS International Project Week and Scientific Conference, Szeged, Hungary, May 6-10, 2019.
- Adsorption of ammonium using pomegranate peel as low-cost adsorbent: International Conference on Science, Technology, Engineering and Economy (ICOSTEE), Szeged, Hungary, 25th October, 2018.
- Adsorption of ammonium by pomegranate peel: 4th International Congress Food Technology, Quality and Safety, Novi Sad, Serbia, October 23-25, 2018.
- Agricultural wastes as adsorbent (Poster): Chemical Engineering Conference 2018, Veszprém, Hungary, April 24-26, 2018.