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Re-valorization of Electronic Waste - Moss as a Biosorber for Gold Recovery?

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Abstract

Electronic waste contains high amounts of gold and is therefore an important secondary source of this raw material. While conventional metal recovery processes are associated with environmental and health risks along with high energy demand, bio-based methods represent a sustainable alternative of growing importance. In our previous work, we discovered that the moss Physcomitrella patens not only selectively binds Rare Earth Elements (REEs) but can also bind gold with even higher efficiency. This prompted us to conduct a comparative study on the biosorption of gold by P. patens and two further moss species (Physcomitrium eurystomum and Physcomitrium sphaericum). Sorption capacities from model gold solutions reached 1.2 ± 0.4 mmol g-1, 1.1 ± 0.6 mmol g-1 and 0.8 ± 0.1 mmol g-1, respectively, and were up to 3.7-folds higher than these obtained for selected REEs. As expected, the efficiency of gold removal was affected by the pH of the metal solution. Furthermore, reduction of gold ions seems to play an important role in the gold recovery by mosses. Thus, although further studies on the mechanisms of gold recovery by moss species are needed, the work presented here provides important insights into the use of moss for the re-valorisation of e-waste as a secondary source of gold.

1 Introduction

With the increasing use of electronic devices and the development of new technologies, a considerable amount of electronic waste (e-waste) is produced. While annual global e-waste production is continuously increasing (from 44.7 million tons in 2016 to 53.6 million tons in 2019), still only about 20–30% of it is recycled (Guha, 2021). At the same time, due to the high content of precious metals in comparison to primary natural resources, e-waste is an important source of secondary raw materials, such as gold, platinum, palladium, and rare earth elements (REEs) (Hsu et al., 2019; Işıldar et al., 2019). For example, about 250 g of gold is contained in a tonne of printed circuit boards used in typical personal computers which is 25 to 250 times the gold content found in average gold ores (Abdelbasir et al., 2018). Thus, recovering critical precious metals from e-waste is crucial, not only to cover the growing demand but also to support the concept of circular economy and sustainable development.

Whereas REEs, classified by the European Commission among 27 critical raw materials (European Commission Communication 2017), are needed for devices and applications for high-end technology and sustainable energy (Giese, 2020), gold is also of great economic importance. Although it is mainly used for jewelry production and in the investment sector, due to its high electrical conductivity and chemical inertness, gold electrodes are necessary for many technological applications, especially in electronic systems and consumer electronics. Furthermore, gold nanoparticles are used in detectors as well as catalysts and their significance in medical applications, e.g. for cancer treatment, is constantly increasing (Zhu et al., 2019). Finally, alongside with tin, tantalum and tungsten gold belongs to so called conflict minerals for which responsible acquisition and supply chains are regulated by the European Union (EU, 2017).

The techniques that are commonly used for metal recovery from e-waste lack selectivity, have high energy demand, utilize hazardous chemicals and produce toxic fumes (Jadhao et al., 2021). In contrast, bio-based methods employing (micro)organisms are environmentally friendly and can be an efficient alternative for such purposes (de Freitas et al., 2019; Işıldar et al., 2019; Jadhao et al., 2021; Shamim, 2018). While bioleaching enables the extraction of metal compounds from solid particles, biosorption allows the selective binding and pre-concentration of the dissolved metals on the surface of biomass. It is considered as economical, simple, and sustainable alternative for the recovery of metals from waste solutions to hydrometallurgical processes (Giese, 2020). In general, several different mechanisms can be responsible for biosorption processes, such as physical (electrostatic or Van der Waals) interactions, ion exchange, chelate formation, reduction, precipitation and complex formation (Kanamarlapudi et al., 2018). Furthermore, different factors such as biosorber selection and its characteristics (e.g. functional groups at its surface), the sorptive agent, the pH of the solution, contact time, temperature, the pretreatment of the biomass used or the ratio of the adsorber to adsorbate influence the efficiency of this process (Derco & Vrana, 2018; Kanamarlapudi et al., 2018; Park et al., 2010).

In our previous studies, we identified a moss species – *Physcomitrella patens* – as an interesting candidate for the use as biosorber for recovering REEs (Heilmann et al., 2021; Heilmann et al., 2015). We showed that *P. patens* not only binds REEs selectively from dilute solutions (e.g. with a maximum sorption capacity for neodymium of $Q_{max,Nd3+}$ = 0.74 ± 0.05 mmol per gram cell dry weight) but can also bind gold with even higher efficiency ($Q_{max,Au3+}$ = 1.59 ± 0.07 mmol g^{-1})(Heilmann et al., 2021). This remarkable result prompted further investigations of biosorption by *P. patens* and two other moss species (*Physcomitrium eurystomum* and *Physcomitrium sphaericum*). In this study, the focus was on an in-depth characterization of gold sorption – not only in dilute model solutions of different compositions but also in real bioleachates and as a function of biomass pre-treatment. For a comparative classification of the maximum sorption capacities, additional experiments were carried out with two representatives of the REEs, namely terbium (Tb^{3+}) – classified as heavy REE – and neodymium (Nd^{3+}) – which belongs to light REEs.

2 Material And Methods

Chemicals

All chemicals used in this study were of analytical grade. For sorption experiments aqueous solutions of gold (tetrachloroauric(III) acid, $HAuCl_4$ * 3 H_2O, ≥ 99.5%, Carl Roth, Karlsruhe, Germany), neodymium (neodymium(III) nitrate, $Nd(NO_3)_3$, 99.9%, Sigma-Aldrich, St. Louis, USA) and terbium (terbium(III) nitrate, $Tb(NO_3)_3$ * 5 H_2O, 99.9% Sigma-Aldrich, St. Louis, USA) were used. Salts needed for preparation of the Knop medium used for moss cultivation were either purchased from Merck (Darmstadt, Germany) (K_2HPO_4, $MgSO_4$, $FeSO_4$) or from Carl Roth (Karlsruhe, Germany) ($NaHCO_3$, KCl, $Ca(NO_3)_2$). Standards used for quantitative analysis of terbium (Tb standard for inductively coupled plasma-mass spectrometry

(ICP-MS)), neodymium (Nd standard for atomic absorption spectroscopy (AAS)) and gold (5.1 mM solution in 5% HCl) were purchased from Sigma-Aldrich (St. Louis, USA).

Determination of metal concentrations

The quantification of the selected REEs from aqueous solutions containing only one metal species was performed via a spectrophotometric assay employing the color reaction with xylenol orange (XO, Fluka, Buchs, Switzerland) as described previously by Heilmann et al. (2015), using terbium and neodymium standards for calibration and a multilabel reader (EnSpire 2300, PerkinElmer Inc., USA) for measurements. For the determination of gold either a graphite furnace atomic absorption spectrophotometer (GF-AAS, SIMAA 6000, Bodenseewerk Perkin-Elmer GmbH & Co. KG, Überlingen, Germany) or an inductively coupled plasma-mass spectrometer (ICP-MS, iCAP Q, ThermoScientfic, Waltham, USA) was used.

For AAS measurements 20 µL of the sample were mixed automatically with 5 µL of matrix modifier (aqueous mixture of 2.1 mM magnesium and 9.4 mM palladium), injected into the graphite furnace, heated according to the optimized temperature protocol and atomized (**Supplementary Information, Table A**). The measurement was performed at the wavelength of 242.8 nm and 10 mA was applied to the hollow-cathode lamp for gold determination (IntensitronLamp, PerkinElmer Inc., Waltham, USA). A gold standard was used for calibration and all dilutions were performed with 0.2% (w/v) solution of nitric acid (≥ 65% *puriss. p.a.*, Carl Roth, Karlsruhe, Germany) in 2-mL sample vials (polystyrol, BRAND, Wertheim, Germany). The limit of quantification (LOQ) for the GF-AAS gold determination was 0.3 µM.

Solutions containing both Tb and Nd as well as samples from sorption experiments with bioleachate (see section **Recovery of gold from bioleachate**) were subjected to ICP-MS determination, which was kindly provided at the facility of BRAIN AG (Zwingenberg, Germany). In these cases, the samples were mixed with aqueous carrier solution of 0.5% (v/v) HCl and 2% (v/v) HNO_3 (Carl Roth, Karlsruhe, Germany). The limit of detection (LOD) of ICP-MS measurements were 0.25 nM for Au, 0.35 nM for Nd and 0.31 nM for Tb.

Moss species and their cultivation

Moss species used in this work were obtained from the International Moss-Stock-Center (IMSC, Freiburg, Germany). Three moss species: *Physcomitrella patens* (IMSC #40001), which was identified as very good biosorber of REEs and gold in our previous work (Heilmann et al., 2021), *Physcomitrella eurystomum* (IMSC #40052), *Physcomitrella sphaericum* (IMSC #40018), two further representatives of the Funariaceae family, were selected for the sorption studies. Moss species were maintained by cultivating them in 100-mL Erlenmeyer flasks with 20 mL of modified Knop medium on an illuminated shaker (Type AK 82, Infors HT, Bottmingen, Austria) at room temperature (RT) and under atmospheric concentration of CO_2. The cultivation medium consisted of 1.44 mM K_2HPO_4, 3.35 mM KCl, 1 mM $MgSO_4$, 6.1 mM $Ca(NO_3)_2$, and 0.45 mM $FeSO_4$ at a pH of 5.8. The shaking speed was set to 110 revolutions per minute (rpm) and the average photosynthetic photon flux density (PPFD) of the LED-tube (L18W/840, Osram,

Munich, Germany) was 40 µmol m^{-2} s^{-1}. Medium exchange was performed every two weeks together with biomass homogenization (T18 Basic Ultra Turrax IKA, Staufen, Germany). Precultures for photobioreactor cultivations (á 200 mL culture volume) were performed in 1-L Erlenmeyer flasks (25°C, 40 µmol m^{-2} s^{-1}, 110 rpm) using 10 mL of homogenized moss culture for inoculation and a regular medium exchange (every two weeks). After eight weeks the biomass was homogenized and transferred into 1-L bubble columns. Each moss species was cultivated in two separate photobioreactors filled with 900 mL of the modified Knop medium, as described above. For pH-stabilization 3.5 mL of a 1 M aqueous solution of $NaHCO_3$ were added to each reactor. Bubble column cultivations were performed over two weeks with an aeration rate of 0.5 L min^{-1} with 3 vol.-% of CO_2 at 25°C and a PPFD of 80 µmol m^{-2} s^{-1}. The biomass was harvested by centrifugation at 3,000 g, room temperature (RT), 10 min and the culture supernatant was discarded. After freezing (24 h at -20°C followed by 24 h at -80°C) the biomass was freeze dried (Alpha 1–4, Christ Osterode am Harz, Germany) for at least 48 h.

Determination of sorption capacities of moss species for gold and selected REEs

Maximum sorption capacities for gold, neodymium and terbium from single- and two-metal-solutions (Au/Nd or Au/Tb) were determined for the biomass of *P. patens*, *P. eurystomum* and *P. sphaericum*. For this purpose, the lyophilized moss biomass was grinded with mortar and pestle until a homogeneous powder was obtained. The moss powder was then weighed into 15-mL centrifuge tubes using a microbalance (ABS 220-4, Kern, Balingen, Germany). Next, the biomass was pretreated in a three-stage washing process. First, 2 mL ultrapure water (H_2O_u) were added and the moss suspension was incubated for 1 h at 150 rpm and 21°C (New Brunswick Innova 44/44R Shaker, Eppendorf, Hamburg, Germany). After centrifugation (10 min, 3,000 g, 21°C) (Centrifuge 5810 R, Eppendorf, Hamburg, Germany) the supernatant was discarded and the second washing step performed with 2 mL of a 17.1 mM NaCl-solution (Shaker, 24 h, 150 rpm, 21°C). Finally, step one was repeated and the biomass was weighed again, in order to determine the liquid residues.

For the determination of the sorption capacity of the biomass, 10 mM aqueous solutions of $Nd(NO_3)_3$, $Tb(NO_3)_3$ and $HAuCl_4$ were prepared and the pH was adjusted to 5 using 1 M HCl or NaOH. Each sorption experiment was performed at least in triplicate with 6 mg to 60 mg biomass and 2 to 3 mL of the respective sorption solution per sample. Different biomass amounts were chosen in order to verify whether the same sorption capacities could be reached. Blind samples contained the same volume of ultrapure water (H_2O_u) instead of the sorption solution and were included in each experimental set up. Furthermore, control samples containing the respective sorption solution but no biomass were also prepared along. All samples were incubated for 24 h at RT under stirring. The suspension was centrifuged (10 min, 10,000 g, RT) and 1 mL of the supernatant was removed for the determination of metal concentrations and stored in 1.5-mL sample vials (PP, Sarstedt, Nümbrecht, Germany) until analysis. The quantification of the respective metal ions was performed as described above. The sorption capacity (Q) was calculated according to **Eq. 1**:

$$Q_i = \frac{n_{i,0} - n_{i,f}}{m_{BMd}}$$

where $n_{i,0}$ corresponds to the initial amount of the respective element, $n_{i,f}$ to the amount of the element remaining after the incubation and m_{BMd} to the mass of the dried biomass used.

Biosorption of gold from solutions with various pH

In order to examine the influence of the pH on the efficiency of gold sorption, experiments using the biomass of *P. patens* and gold solutions with pH values ranging from pH 3 to 9.2 were performed. About 10 to 20 mg of the biomass powder were weighed into 15-mL centrifuge tubes and the biomass was washed using the above-described procedure. A gold stock solution (11.8 mM) was prepared by solving $HAuCl_4$ in H_2O_u and diluted for each experiment. For the experimental series a concentration of 10 mM was chosen and the individual pH values of 3, 4, 5, 6, 7 and 9.2 were adjusted by using 1 M / 0.1 M NaOH or 1 M / 0.1 M HCl, respectively. The experiments with the respective sorption solution (3 mL) were carried out using three samples with roughly 10 mg and further three samples with about 20 mg biomass. In addition, blind and control samples were prepared as described above. All samples were incubated for 24 h at 150 rpm at 21°C (New Brunswick Innova 44/44R Shaker, Eppendorf, Hamburg, Germany) and centrifuged for 10 min (10,000 g, 21°C). 1.5 to 2 mL of the supernatant were filled in sample vials for further analysis. The pH-value after the sorption experiment was measured and the determination of the gold concentration was performed via GF-AAS.

Recovery of gold from bioleachate

In order to test the removal of gold from a solution that can be obtained during one of the e-waste recycling steps, experiments with bioleachate (40 µM Au^{3+}, pH 9.2) provided by the BRAIN AG were performed. The dried, homogenized and washed biomass of *P. patens*, *P. eurystomum*, and *P. sphaericum* (10–40 mg) was used and blank and control samples were prepared along as described above. After the adjustment of the pH of the bioleachate to pH 5 using 1 M HCl, 2 mL of the solution were added to the biomass and incubated for 24 h (RT, magnetic stirrer). After incubation, the samples were centrifuged (10 min, 10,000 g, RT) and 1 mL of the supernatant was transferred to a 1.5-mL sample vial (PP, Sarstedt, Nümbrecht, Germany) for further analysis. The supernatants were diluted with the carrier solution and measured with the ICP-MS as described above.

Gold removal using fresh moss biomass

Beside the sorption experiments with dried, lyophilized and washed biomass, sorption of gold by freshly harvested *P. patens* biomass was investigated over a period of 7 days. For this purpose, about 20 to 70 mg of the wet biomass were weighed into six 100-mL Erlenmeyer flasks filled with 20 mL of modified Knop medium. Three of the flasks were used as growth controls, while the other three were spiked with 10 mM $HAuCl_4$ solution (pH 5) to reach a gold concentration of 60 µM on day one. Four days after the first spiking, a second gold spike was added. Three additional flasks with 20 mL medium spiked with 60 µM

gold on the first day of the experiment but without biomass were used as negative controls. All flasks were placed on an illuminated shaker (110 rpm, Promax2020, Heidolph, Schwabach, Germany) at RT and atmospheric CO_2 concentration during the experiment. Every 24 h, 200 µL of the supernatant were withdrawn from each flask to determine the gold concentration using the GF-AAS as described above.

3 Results

Cultivation of the selected moss species

All three selected moss species – *P. patens, P. eurystomum* and *P. sphaericum* – were cultivated under the same conditions according to a two-stage protocol: The biomass obtained from a 200 mL shake flask culture grown over an 8-week period was further expanded in two bubble columns with working volumes of 900 mL for 2 weeks. Compared to the shake flasks, the cultivation conditions in the bubble columns were intensified by CO_2 enrichment of the supply air to 3 vol-%, Na_2CO_3 addition to the medium for pH stabilization and an increase of the PPFD from 40 µE to 80 µE. Due to the filamentous nature of the moss species, the determination of biomass concentrations and the recording of growth curves is not trivial. Therefore, the final yield of freeze-dried biomass at the end of the cultivation was primarily used for growth comparison, as summarized in **Table 1**. The biomass obtained with *P. patens* was the highest, followed by *P. eurystomum* and *P. sphaericum*. In this context it is important to note that the biomass of *P. patens* was harvested from bubble column reactor 2 (BCR 2) two days earlier due to clogged aeration nozzles resulting in a 16% lower final biomass. This fact further emphasizes the slight growth advantage of *P. patens* over the other two moss species.

Sorption capacities: single-component solutions

Sorption experiments with solutions containing a single metal ion were performed using dried biomass of *P. patens*, *P. eurystomum*, and *P. sphaericum*. Solutions of tetrachloroauric(III) acid, neodymium or terbium nitrate (2–3 mL, 10 mM, pH 5) were incubated with 6 to 46 mg of the respective biomass (24 h, RT, n = 6) and sorption capacities (Q) were calculated according to **Eq. 1** (Fig. 1, **Supplementary information Tables B-D**).

The highest sorption capacities, which reached almost 2 mmol g^{-1} in individual measurements, were obtained for gold, regardless of the moss species used. With 1.2 ± 0.4 mmol g^{-1} determined for *P. patens*, 1.1 ± 0.6 mmol g^{-1} for *P. eurystomum* and 0.8 ± 0.1 mmol g^{-1} for *P. sphaericum*, these values were up to 3.1- and 3.7-times higher, respectively, than the values reached for terbium or neodymium by individual species. While the highest Q-value for terbium (0.49 ± 0.05 mmol g^{-1}) was achieved when using the biomass of *P. patens*, the sorption capacities for neodymium ranged from 0.26 to 0.37 mmol g^{1} with no statistically significant difference between the applied biosorbers. Relating the values of the two selected REEs for the individual moss species, a slight but statistically non-significant trend towards better binding of terbium compared to neodymium was observed.

While all three moss biomasses showed similar patterns with respect to their sorption capacities for the selected elements ($Q_{Au} > Q_{Tb} \approx Q_{Nd}$), the biomass of *P. patens* always seemed to perform slightly better.

In each case the highest sorption capacities with Au from single metal solutions ($HAuCl_4$) were reached for the lowest amount of the biomass of the respective moss species (**Supplementary Information, Table B**). The use of higher amounts of biomass did not lead to a proportionally higher removal of gold from the solution. For example, increasing the biosorber by a factor of approximately 5 improved the relative gold removal for *P. patens* by only 21% (56% removal by 7 mg dried biomass in comparison to 77% by 33 mg) while for *P. eurystomum* no improvement at all could be observed (63% Au removal at 6.4 mg biomass compared to 62% at 34.5 mg). For the REEs, no clear correlation between biomass amount and metal removal was observed.

Sorption capacities: two-component solutions

When either a combination of gold and terbium or of gold and neodymium solutions (5 mM of each metal ion, pH 5, 2 mL) was incubated with the respective moss species, the removal of the REEs seemed not to be affected by the presence of the gold ions, as the sorption capacities obtained for the REE representatives and the corresponding species were not statistically significantly different from those calculated in the first experiment (**Table 2**). This implicates that different mechanisms are responsible for the removal of gold and terbium/neodymium. Again, slightly higher Q-values, however statistically significant only in case of neodymium and *P. eurystomum*, were observed for *P. patens*. Although the mean values for gold sorption capacities calculated for the two-component solutions were lower than those from the single gold solution, this obviously resulted from the lower amount of gold present in the mixture compared to the single-metal solutions, as the removal reached over 90% in most cases (**Supplementary Information Table E and F**). Even though the higher sorption capacities for gold than for REEs determined in this work suggest a higher affinity of the investigated biomass for the first metal, based on this data no selectivity of the biosorber for Nd/Tb and Au can be assumed.

Influence of the pH of gold solutions on biosorption

As the pH of a metal solution can influence the efficiency of biosorption, the effect of different pH values between 3 and 9.2 was examined when using the biomass of *P. patens*. For this purpose, gold solutions (10 mM $HAuCl_4$, 3 mL) with adjusted pH values were incubated with the dried biosorber material (10–20 mg) for 24 h (RT). Subsequently, the concentration of gold in the supernatant was quantified by AAS as described above and the sorption capacity was calculated according to **Eq. 1**. As shown in Fig. 2, the highest average sorption capacity was registered for the solution with pH 6 (1.24 ± 0.05 mmol g^{-1}), which was about 1.5 times higher than the values obtained for the solutions of pH 4 and 5 and over 7 times higher than the mean value obtained at pH 9.2. At neutral pH, the calculated sorption capacity dropped to 0.37 ± 0.08 mmol g^{-1}, thus showing more than threefold lower removal efficiency than at pH 6. The high standard deviation of the Q-values is mostly due to the measurement uncertainty, especially when about 10 mg of the biomass was used, as the differences between initial and end gold concentration in the

supernatant were only small (**Supplementary Information Table G**). While blind and control samples (biomass mixed with water and corresponding gold solutions without biomass) showed no discoloration after the incubation, the supernatant of gold solutions incubated with the biomass turned purple, with the most intense color observed at pH 6 and the least at pH 3 and 9.2. The biomass of these samples also showed dark purple to black coloration, whereas the biomass incubated with ultrapure water remained green (See **Supplementary Information Figure A**). Furthermore, golden, line-shaped deposits could be observed on the inner wall of the vials. All in all, this suggests that gold reduction is involved to some extent in the process of gold removal from the metal-containing solution.

Gold recovery with moss biomass from bioleachate

Biosorption experiments with bioleachate solutions supplied by BRAIN AG were performed in order to investigate gold recovery from such complex solutions, which ultimately represent the real sources for biorecovery processes. The used bioleachate of an industrial recycling material (39.2 µM of gold) with an original pH of 9.2 was adjusted using 1 M HCl to a pH of 5 and incubated with dried biomass of all three moss species. The results are summarized in Table 3. The sorption capacities calculated for gold from the bioleachate were only around 1 µmol g^{-1}. Moreover, no statistically significant differences could be observed for the three different moss species. The lowest concentration measured in the bioleachate solutions after the incubation with the biomass accounted to approximately 25 µM (**Supplementary Information Table H**). In additional experiments with defined $HAuCl_4$-solutions in the lower millimolar range this final concentration was measured with a starting value of 1.14 mM Au^{3+} resulting in a sorption capacity of Q = 0.23 mmol g^{-1} (data not shown). This points to the fact that the bioleachate contained additional components and metal ions that probably interfered with the biosorption process. Additionally, it is also possible that the process parameters used for gold recovery from bioleachate, such as the incubation time, the pH of the solution, biomass pretreatment and amount, temperature, etc. need to be optimized for this complex solution in order to reach a higher efficiency. Although the obtained sorption capacities for the removal of gold from bioleachate were significantly lower compared to the results with model solutions, about 30% of the target metal could still be recovered from the sample, which is a good starting point for further investigations.

Gold removal using moss biomass without pre-treatment

In addition to the biosorption experiments with dried and washed moss biomass, the possibility of gold recovery from metal-containing solution using the biomass of *P. patens* harvested from the preculture and resuspended in culture medium was also investigated. For this purpose, the collected moss biomass was weighed into three Erlenmeyer flasks and filled with Knop medium. Gold solution ($HAuCl_4$) was then added to the flasks at t = 0 h and again after 72 h. Three control flasks, containing the same medium but no biomass, were also spiked, but only once at 0 h with the same amount of the gold solution, to monitor possible gold removal without biomass involvement. Samples of the supernatant were taken from each flask in 24-h intervals and submitted to Au-quantification via AAS.

As depicted in Fig. 3, the gold concentration in the flasks without moss biomass decreased slowly and reached approximately half of the initial concentration (65 ± 2 µM) within 7 days of the experiment, while it was removed much faster and more efficient from the solution in the presence of the moss biomass. After six days of incubation, yellowish-gold particles could be observed on the medium surface of the control samples. Here, a connection between the reducing Fe^{2+} ions in the Knop medium and the observed reduction of the gold can be suspected. As a result, formed elemental nano-gold particles were floating on the surface of the solution or precipitated out of the medium over time and were not detected in the collected medium samples. For comparison, in the flasks with moss biomass, more than half of the gold was removed from the supernatant already after the first 24 h, reaching the limit of detection (LOD = 0.03 µM) in two out of three flasks and thus the mean removal of about 95% after 72 h. Interestingly, also after the second spiking with gold solution mean removals of more than 63% and 85% were reached after 24 and 72 h, respectively. The biomass in the test flasks turned reddish to reddish-brown already one day after spiking, while additionally prepared growth controls (without gold addition) remained green throughout the experiment. Moreover, the surface of the culture medium was covered with a light purple film and no visually detectable growth of the moss was observed in the test flasks, indicating toxic effects on *P. patens*. The color change of the biomass and the solution suggests the death of the moss and a reduction of gold ions during the experiment.

After the experiment, the loaded biomass was collected from the test flasks, the gold was extracted with *aqua regia* and determined via ICP-MS. The biomass samples (22, 32 and 68 mg of wet weight) contained 1.2, 1.7 and 2 µmol gold, corresponding to 55, 53 and 29 µmol g^{-1}, respectively. As the extraction with *aqua regia* is quantitative, apparently 40 to 60% of the amount of gold originally added to the medium was recovered. On the other hand, gold was detected in the supernatant in only one of the three flasks at the end of the sorption experiments, so the expectation was that nearly 100% had been taken up by the biomass and should be extracted again. We hypothesize that some of the gold ions and reduced Au-species adsorbed on the surface of the culture flask or settled on the surface after reduction, so that no gold was found in the supernatant. However, further investigations are needed to test this hypothesis.

4 Discussion

The moss species used in this study, most notably *Physcomitrella patens*, are known to grow up to three times faster than typical suspension cultures of higher plants (Hohe & Reski, 2005). The growth of *Physcomitrella patens* in different reactors has already been successfully optimized in several studies (e.g (Cerff & Posten, 2012; Chodok et al., 2010)). Important parameters are the quality and intensity of the light used for illumination, aeration rate, CO_2 concentration, temperature and pH of the medium. Through optimization, biomass concentrations of up to 3.6 g L^{-1} (dry weight) were achieved in shake flasks after 14 days cultivation time (Chodok et al. 2010), which is much higher than the values reached after bubble column cultivation in this study (up to 1.48 g L^{-1}). It is therefore to be expected that the biomass yield of moss species can also be significantly improved in bubble column cultivation. However, an aspect that

should not be neglected in this context is that the sorption capacity of the biomass for a given element might not be independent of the cultivation conditions (Heilmann et al., 2021). For example, the sorption capacity determined here for neodymium and *P. patens* (Q_{Nd} = 0.37 ± 0.14 mmol g^{-1}) corresponds well to the values previously determined for this moss from the shake flask cultivation (Q_{Nd} = 0.28 ± 0.03 mmol g^{-1}), while it is lower than the values obtained for the biomass of *P. patens* from an airlift reactor with internal LED-illumination (Q_{Nd} = 0.74 ± 0.05 mmol g^{-1}) (Heilmann et al., 2021). Thus, the target function of an optimization should not be the biomass itself but the total sorption capacity per liter culture volume.

Due to different experimental setups, a comparison of gold sorption efficiencies for the species investigated here with data reported previously in peer-reviewed literature for various biosorbers is quite difficult. For example, Nakajima (2003) tested fresh biomass of 30 different microorganisms (bacteria, actinomycetes, fungi and five yeast species) for their gold accumulation potential in three different gold solutions. For the solution of tetrachloroaurate(III) (50 µM, pH 3, 1 h, 30°C) the highest values of 0.34 mmol g^{-1} and 0.33 mmol g^{-1} were reached using *Pseudomonas maltophilia* and *Escherichia coli*, respectively. Higher gold sorption capacities (335 mg g^{-1} corresponding to 1.8 mmol g^{-1}) were reported by Ji et al. (2011) for dried biomass of *Bacillus subtilis* at an initial gold concentration of 80 mg L^{-1} (406 µM). Non-living biomass of brown seaweed *Sargassum natans* showed a gold sorption capacity of 420 mg g^{-1} (corresponding to 2.13 mmol g^{-1}) at an initial gold concentration of 5 mM, pH 2.5 and 16 h (Kuyucak & Volesky, 1988). Nevertheless, still very little is known about gold sorption capacities of moss species as the only data published so far concern our previous studies with *P. patens* (Heilmann et al., 2021), which are in good agreement with the values presented here.

The experiments conducted with two-component metal solutions suggest that different mechanisms are responsible for the removal of gold and terbium/neodymium since the sorption capacities appeared to be independent of each other. However, further studies on the biosorption mechanisms of the respective target elements are needed to obtain a clearer picture. A look at mechanisms that have been described for the sorption of those elements by other organisms may be helpful here. Maeda and Tsuruta (2020) studied microbial gold biosorption in detail and discovered a two-stage process: A small amount of gold is removed from solution within the first hours by biosorption until an equilibrium state is reached. A greater amount of gold(III)-ions is reduced afterwards to zero-valent gold. For example, the gold (III) removal process using *Candida krusei* reached the first equilibrium after about 10 h and increased again after 22 h, accompanied by a dark coloration of the solution which indicates a reduction process (Maeda & Tsuruta, 2020). Similar to this, in all cases where the moss biomass was incubated with a gold solution, light to dark purple coloration of the supernatant, characteristic of colloidal gold, was observed after 24 h, thus indicating that reduction processes also play a role in gold removal using moss species.

Variation of the pH of the gold solution revealed that the removal was best at acidic pH between 4 and 6, while lower and higher pH values were less favorable to the process. This fits well with the postulate formulated by Shamim (2018) for heavy metals, stating that pH values from 2.6 to 6 are generally

advantageous for biosorption processes. The pH effect can possibly be attributed to various factors. Nakajima (2003) described that in a solution of hydrogen tetrachloroaurate(III), which was also used in our experiments, the composition of gold ion complexes strongly depends on the pH. While at pH 1 the dominating gold species is $AuCl_4^-$ (ca. 97%), the chloride ions in the complex are displaced by $(OH)^-$ groups as the pH of the solution increases. At pH 6 $Au(OH)_4^-$ complexes account for more than 90%. It has also been postulated that when Au(III) binds to biomass, chloride ions are displaced from the complex by the ligand groups of the cells, and therefore the complex composition of the solution affects the biosorption process. While in Nakajima's studies, in which cells of *Pseudosomonas maltophilia* were used, the highest accumulation of gold was determined at pH 2 and 3, where gold complexes with four, three and two chloride ions are the main components, the highest value for the moss biomass was obtained at pH 6. This contrasting observations could be due to different ligands and their different composition present in bacteria and mosses used in both studies, as well as different mechanisms that are overlaying gold removal when using these biospecies.

The color change from green to purple / black of the moss surface registered after the incubation with gold solution, indicates either changes in the moss surface or an accumulation of gold nanoparticles on the biosorber. Lengke et al. (2006) described the mechanisms of gold bioaccumulation from $HAuCl_4$ solutions by cyanobacteria (*Plectonema boryanum*) via spontaneous formation of nanoparticles of amorphous gold (I)-sulfite at the cell walls and subsequent deposition of metallic gold near the surface and in solutions. Similar observation were made by Chakraborty et al. (2008) who investigated biorecovery of gold by other cyanobacteria (*Lyngbya majuscula, Spirulina subsalsa*) and an eukaryotic alga (*Rhizoclonium hieroglyphicum*). The authors describe different mechanisms that occur during the incubation of these species with gold(III) solution such as initial adsorption on the surface of the biomass, followed by active accumulation and reduction of the gold ions. Since the strongest color change of the biomass and the gold solution observed here was detected at pH 6, it seems likely that the contribution of reduction from Au(III) to Au(I) and then Au(0) was the most significant in this case. This increased contribution of the reduction to gold removal from the supernatant at pH 6 may also be a reason for the improved sorption capacity.

The significant reduction of the biosorption capacity for gold in the bioleachates was not unexpected, considering that a large number of other types of metal ions can be expected to be dissolved from the electronic waste. Similar effects have been described in other studies. For example, Tsezos et al. (1996) studied the gold uptake by two bacterial strains (*Pseudomonas sp.* strain BP 7/26 isolated from a silver mine and *Pseudomonas medocina* strain AS 302 isolated from a copper mine) in the presence of palladium, which is also present in e-wastes. The authors concluded that gold and palladium compete with each other, possibly showing a preference for the same binding-groups of the biomass. For these reasons, further analyses of the composition of the bioleachates as well as model studies with defined multicomponent solutions, as already performed with rare earths in this work, are necessary to elucidate competitive binding effects and to effectively design biosorption processes.

5 Summary And Conclusions

The data presented in this work confirms that selected moss species, particularly *P. patens*, have the potential to be used as biosorber for gold recovery. Gold sorption capacities registered for this biomass were up to 3.7-times higher than those obtained for two representatives of REEs. Although, based on the data collected here, no concrete selectivity can be assumed with respect to the target metal ions and the moss species studied, the recovery of gold does not seem to be affected by the presence of neodymium and terbium, and vice versa. However, a sorption experiment performed with a multicomponent bioleaching solution resulted in lower recovery of gold, thus it is possible that other components of bioleachates can affect its removal. Furthermore, various and different mechanisms seem to underlie the recovery process of gold and REEs. Especially in case of gold, the reduction of ions plays a substantial role, however further studies are necessary in order to assess its share and influence on the intended use of moss as a biosorber. Moreover, different efficiencies of gold removal reached for the biomass of *P. patens* at different pH values and concentrations of the gold solutions, show that these parameters have to be carefully considered when developing such biorecovery processes. While the mechanisms of gold recovery by moss species still need to be studied in detail to be fully understood, the work presented here highlights important variables influencing the use of moss for the re-valorisation of e-waste as a secondary source of gold.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Christos Karampatzis, Benedikt Böhm and Anna Maria Becker. The draft of the manuscript was written by Anna Maria Becker, which was commented by all authors. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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Tables

Table 1. Biomass yield of moss cultivations

P. patens, P. eurystomum, and P. sphaericum were cultivated according to a two-stage protocol involving a 200 mL shake flask culture followed by further expansion in two bubble column reactors (BCRs) with working volumes of 900 mL.

Culture	Freeze dried biomass in g	
	BCR 1	BCR 2
P. patens	1.33	1.12[a]
P. eurystomum	1.06	1.07
P. sphaericum	0.92	0.72

[a] The culture was harvested after 12 days due to clogged aeration nozzles.

Table 2. Mean sorption capacities and metal ion removal from two-metal solutions

The dried biomass (BM_d) of *P. patens, P. eurystomum, and P. sphaericum* was weighed into separate vials, incubated for 24 h (RT) with 2 mL of 5 mM solution of either $HAuCl_4$ and $Tb(NO_3)_3$ or $HAuCl_4$ and $Nd(NO_3)_3$ (pH = 5) and the concentration of metal ions in the supernatant was determined either via XO Assay (Tb/Nd) or AAS (Au) (n = 3 for each sample) after centrifugation (10 min, 10,000 g, RT). Sorption capacities were calculated based on **equation 1** and removal based on the initial concentration of metal ions. Mean sorption capacities marked with an asterisk are significantly different from each other (two-sided, independent sample t-test (Excel), $p^* < 0.05$).

Species	Mean Q_{Au} in mmol g^{-1}	Mean removal Au in %	Mean Q_{Tb}/ Q_{Nd} in mmol g^{-1}	Mean removal Tb/Nd in %
	Au^{3+} & Tb^{3+}			
P. patens	0.67 ± 0.26	96 ± 04	0.42 ± 0.17	68 ± 26
P. eurystomum	0.58 ± 0.30	91 ± 11	0.32 ± 0.09	59 ± 27
P. sphaericum	0.56 ± 0.23	93 ± 11	0.28 ± 0.08	56 ± 29
	Au^{3+} & Nd^{3+}			
P. patens	0.59 ± 0.27	94 ± 07	0.43 ± 0.14*	74 ± 15
P. eurystomum	0.49 ± 0.26	84 ± 15	0.26 ± 0.06*	52 ± 19
P. sphaericum	0.48 ± 0.15	85 ± 19	0.36 ± 0.13*	61 ± 15

Table 3. Sorption capacities and removal of gold from biolechate of an industrial recycling material

Mean sorption capacities (n = 6) were obtained for the biomass of *P. patens, P. eurystomum,* and *P. sphaericum* by incubation with a bioleachate of e-waste. Dried biomass (10 - 40 mg) of the respective moss was incubated (RT, 24 h) with 3 mL of bioleachate solution (39.2 µM Au, pH adjusted with 1 M HCl to 5). Gold concentrations after the incubation ($C_{Au\ end}$) were determined in the supernatant after centrifugation via ICP-MS. Q was calculated according to **equation 1**. Gold removal was calculated based on the initial and final gold concentrations.

Biomass	$C_{Au\ End}$ in µM	Q in µmol g^{-1}	Au-Removal in %
P. patens	25 ± 3	0.98 ± 0.42	30 ± 4
P. eurystomum	25 ± 3	1.09 ± 0.47	32 ± 4
P. sphaericum	25 ± 2	1.00 ± 0.31	31 ± 4

Figures

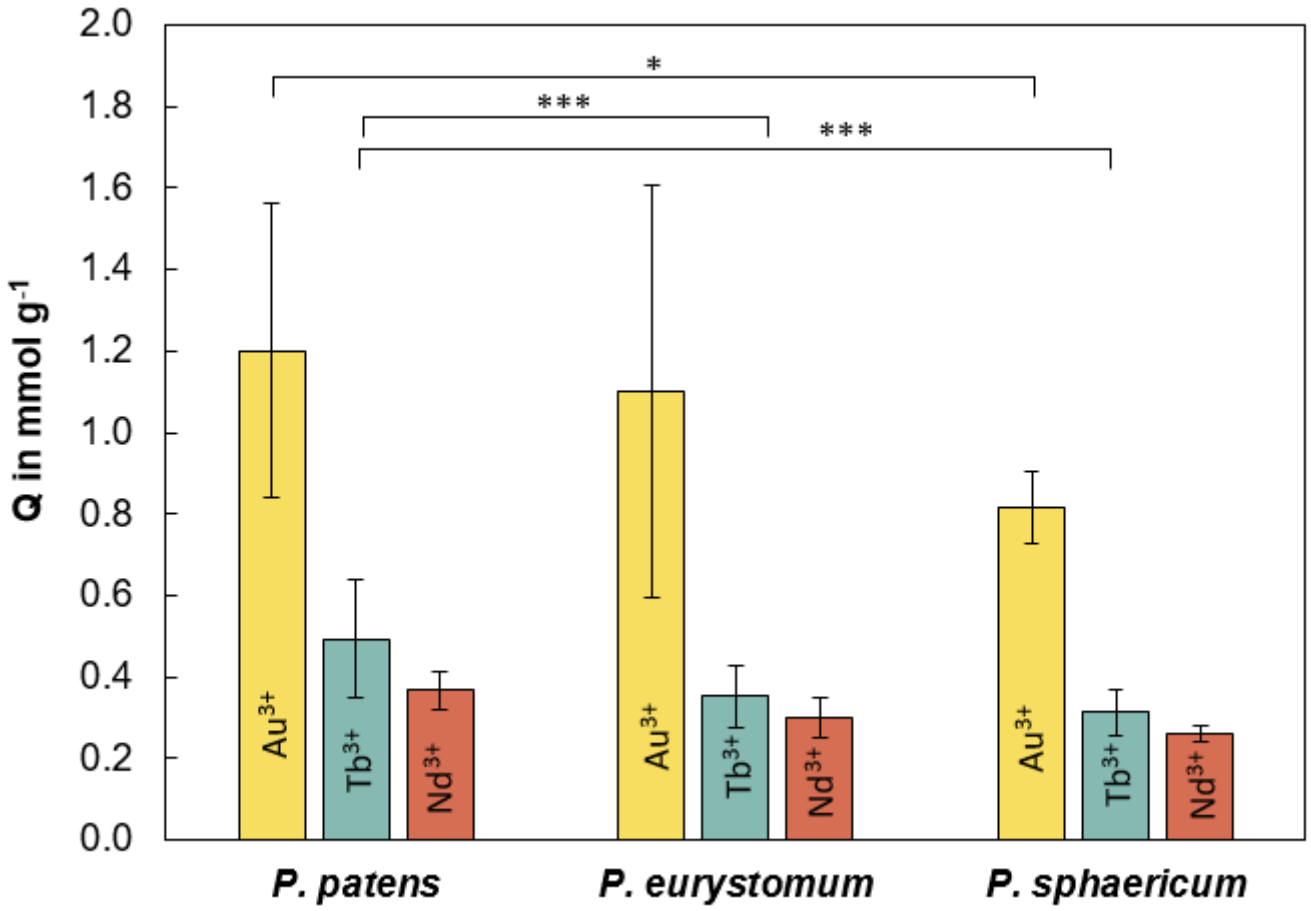


Figure 1

Sorption capacities (Q) of the selected moss species for gold, terbium, and neodymium

Mean sorption capacities (n = 6) were obtained for the biomass of *P. patens, P. eurystomum,* and *P. sphaericum* by incubation with single solutions of gold ($HAuCl_4$, yellow bars), terbium ($Tb(NO_3)_3$, green bars) or neodymium ($Tb(NO_3)_3$, red bars). Dried biomass (6 - 60 mg) was incubated (room temperature, 24 h) in 2 - 3 mL metal solution with 10 mM initial concentration of the respective metal (pH = 5). Metal concentrations were determined in the supernatant after centrifugation via XO-Assay (Tb^{3+} and Nd^{3+}) or AAS (Au^{3+}). Q was calculated according to **equation 1**. Statistical analysis: Pairwise comparisons of sorption capacities were analyzed using two-sided, independent sample t-tests (Excel). Statistically significant differences are marked in the diagram, where * corresponds to $p < 0.05$, ** to $p < 0.01$ and *** to $p < 0.001$.

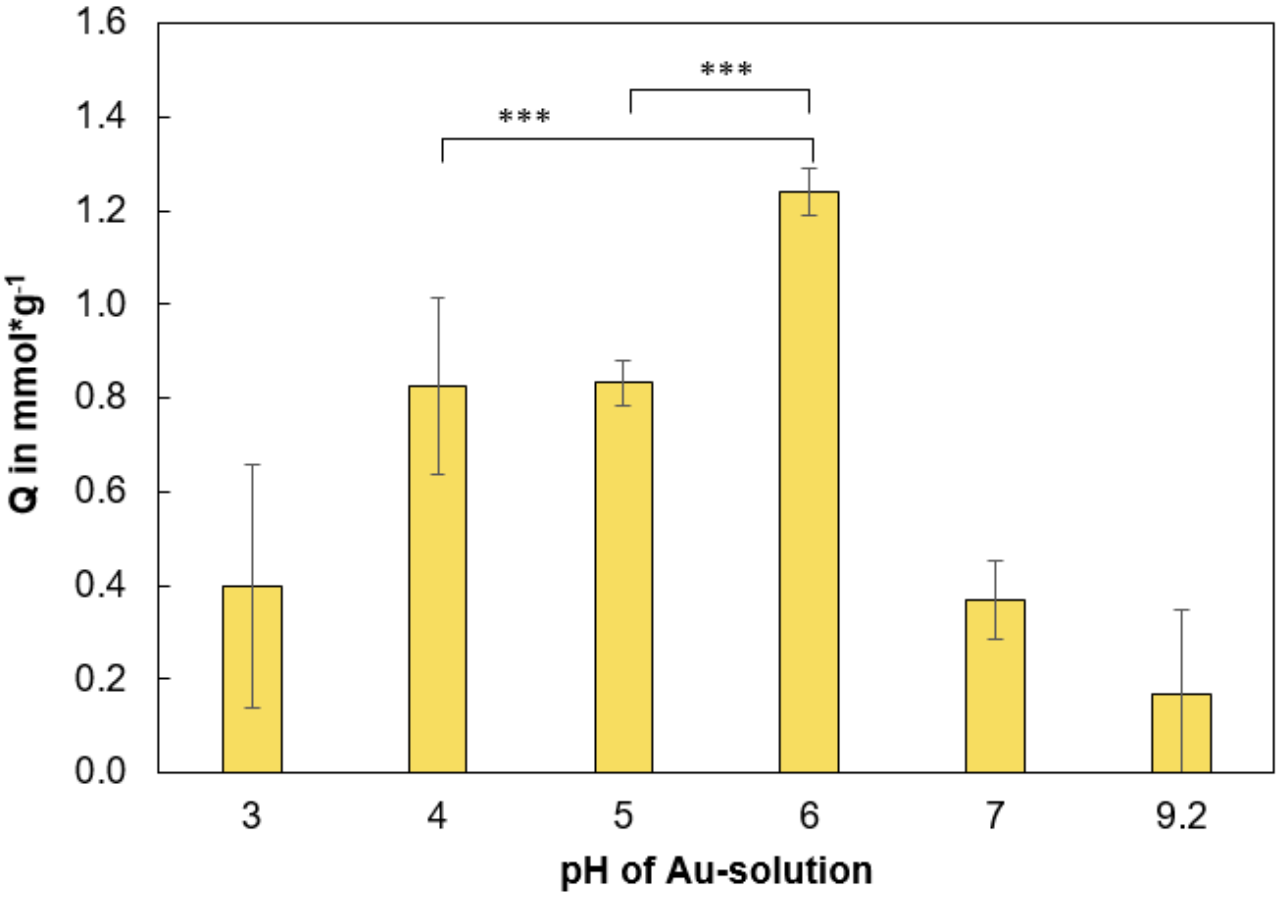


Figure 2

Gold sorption capacities of *P. patens* at various pH

Mean sorption capacities (n = 6) were obtained for the dried biomass of *P. patens* (10 – 20 mg) incubated with 10 mM solution of $HAuCl_4$ (3 mL) with pH between 3 and 9.2. The incubation was performed for 24 h (RT). The gold concentration was determined in the supernatant after centrifugation via AAS and Q was calculated according to **equation 1**. Sorption capacities obtained for the solution with pH 6 were analyzed using two-sided, independent sample t-test (Excel) against those recorded for solutions with pH 4 and 5. Statistically significant differences are marked in the diagram, where *** indicates $p < 0.001$.

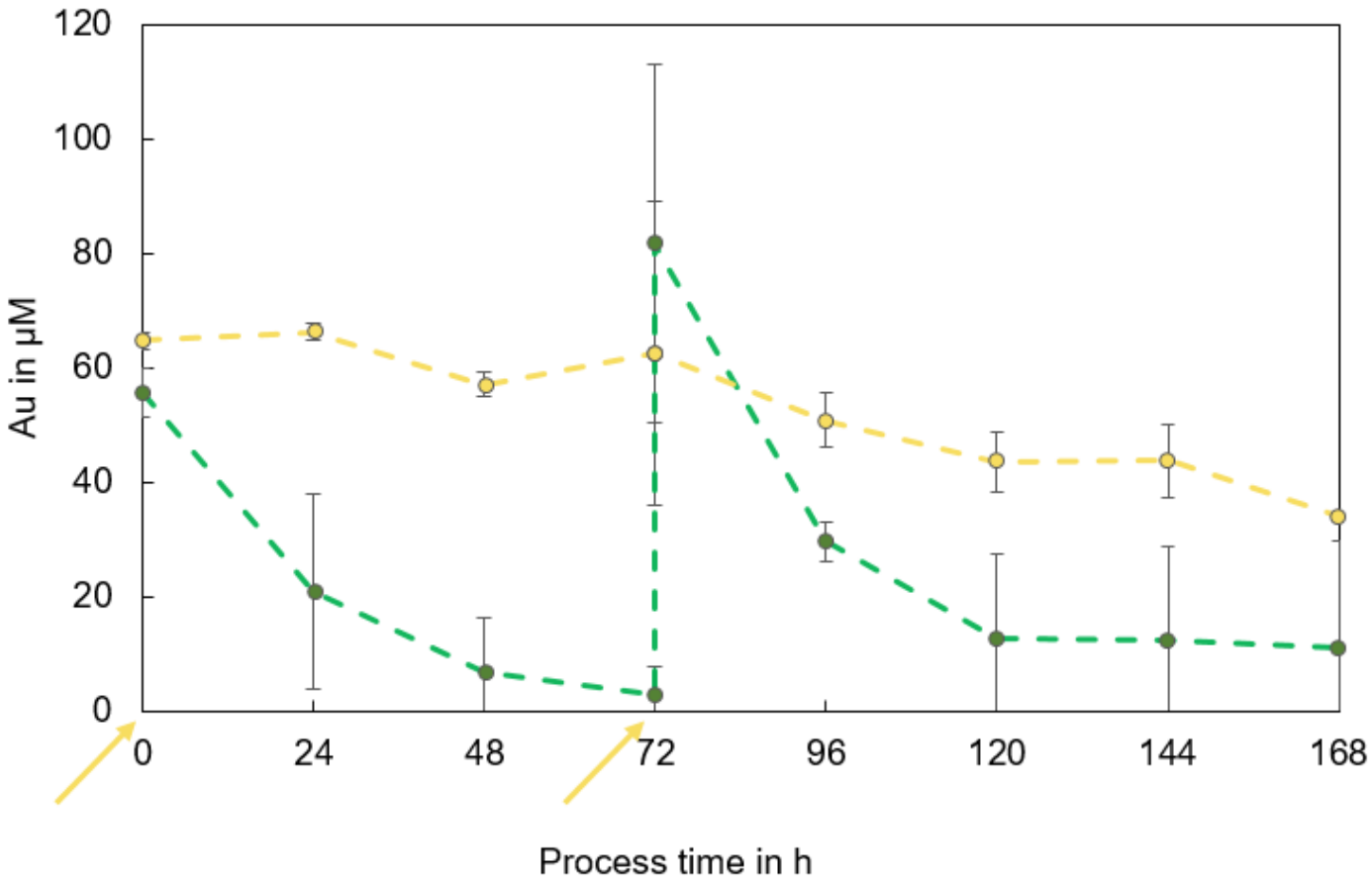


Figure 3

Removal of gold from the culture supernatant by *P. patens*

The concentration of gold in culture medium was monitored over a 7-day incubation with *P. patens*. Dried moss biomass (20 – 70 mg wet weight) was resuspended in 20 mL of Knop medium in Erlenmeyer flasks (n = 3) and spiked with gold solution (160 µL, 10 mM $HAuCl_4$) at t = 0 and t = 72 h (indicated with yellow arrows). Gold control samples were prepared likewise without addition of biomass and second spiking. Supernatant samples (200 µL) were collected from each flask every 24 h and the respective gold concentration was determined via AAS. Each point represents a mean value of the respective gold control (yellow circles) and experimental samples (green circles).

Supplementary Files

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