ENVIRONMENTAL BIOTECHNOLOGY

Preconcentration and detection of mercury with bioluminescent bioreporter E. coli ARL1

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Abstract Cell wall envelopes treated with sodium hydroxide and spray-dried were used as mercury sorbents. The sorbent having sorption capacity 17.7 ± 0.1 μmol/g determined was employed for preconcentration of mercury containing 1– 10 ng/L. After preconcentration, bioavailable mercury was detected in samples of soil, stream, and tap water via induction of bioluminescence of E. coli ARL1. Iron and manganese at concentrations of tenth microgram per liter interfered bioluminescence detection of mercury. In tap water was detected semiquantitatively 0.127 ± 0.1 nmol/L by the induction of bioluminescence of E. coli ARL1 in medium with tryptone after preconcentration using a method of standard addition.

Keywords Mercury detection . Bioreporters . Mercury sorption . Biosorbents

Introduction

Mercury use is widespread, particularly in the production of gold, vaccines, antimicrobials, and electronics. From

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industrial and hazardous waste sites, mercury has seeped into soil, groundwater, and estuaries (Nair et al. [2005\)](#page-8-0). At present, mercury is a ubiquitous pollutant. Mercury cycles through the air, waters, and sediments, changing its form every step of the way. Solid forms, mercury amalgams, are assumed not bioavailable. In most environmental settings, mercury exists as the elemental form Hg^0 , inorganic divalent Hg^{2+} , and organomercury compounds, such as monomethylmercury (MeHg) (Barrocas et al. [2010](#page-8-0)). The geochemical forms of mercury (and subsequent bioavailability) are largely governed by reactions between Hg^{2+} , inorganic sulfides, and natural organic matter. The effect of environmental factors on the availability of inorganic mercury Hg^{2+} to bacteria in aquatic environments are central to human and ecological health concerns with mercury contamination.

Bioluminescent bioreporters are analytical tools for fast and inexpensive detection of bioavailable pollution (Xu et al. [2013](#page-9-0)). Lux strains sensing specific toxic effects have the advantage of being able to respond to mixtures of contaminants inducing the same effect, and thus could be used as a sensor for the sum effect, including the effect of compounds that are as yet not identified by chemical analysis (Woutersen et al. [2011](#page-9-0)). In a case for heavy metal sensor bacteria, expression of a reporter gene is controlled by a metal-responsive regulatory unit, which usually originates from bacteria that are naturally resistant to a particular heavy metal.

The main obstacle of biosensors usage is their low sensitivity, which does not reach levels of mercury contamination in rivers and drinking water (Woutersen et al. [2011\)](#page-9-0). The detection limits were mostly in the microgram per liter range. Two strains, E. coli MC1061(pmerRBSBPmerlux) and Pseudomonas fluorescens OS8(pDNmerRBSBPmerlux) had limit in nanograms per liter (Ivask et al. [2009\)](#page-8-0). In bacteria, chromosomal insertion reduced background luminescence, but this did not lead to higher sensitivity (Woutersen et al.

[2011\)](#page-9-0). The highest sensitivity was found in E. coli HB101(pRB28) immobilized in latex, which gave a detection limit of 0.1 nM HgCl₂ (27.2 ng/L) (Woutersen et al. [2011\)](#page-9-0). E. coli ARL1 harbors a chromosomally inserted 500-bp region of the mer operon consisting of the merR gene and the promoter/operator region of the mer operon fused to the luxCDABE gene cassette of Photorhabdus luminescens (Dahl et al. [2011\)](#page-8-0). Its detection limit for bioavailable Hg^{2+} is approximately 2 μg/L (Dahl et al. [2011\)](#page-8-0).

Recently, we demonstrated the new detection assay with E. coli ARL1 that enabled the detection of 0.57 μ g/L HgCl₂ in diluted artificial sea water (25 % ASW). The sensitive detection was reached by optimization of composition of media for cell cultivation and bioluminiscence induction (Solovyev et al. [2015\)](#page-9-0). Preconcentration of mercury by adsorption on a sorbent is an alternative method of a detection of low concentrations. The sorbent should perform certain selectivity and must not be toxic or inhibit/activated bioluminescence of bioreporters cells. The sorbent must not adsorb light (as e.g., active carbon).These requirements might comply many biopolymers such as chitin, chitosan (Vieira and Beppu [2006\)](#page-9-0), alginate, pectin and its partially deesterified pectic acid derivatives (Synytsya et al. [2007\)](#page-9-0), bacterium biofilm (Rezaee et al. [2008](#page-8-0)), the biomass of green and bluegreen algae (Inthorn et al. [2002\)](#page-8-0), the biomass of aquatic plants (Lacher and Smith [2002\)](#page-8-0), moss (Sari and Tuzen [2009](#page-8-0)), sawdust and pretreated microorganisms. Yeasts possess a potential for accumulating a range of metal cations, and large amounts of these metals can remain associated with the yeast cell wall (Patzak et al. [1997\)](#page-8-0). Yeast cell wall can be used as very effective biosorbent of heavy metals for remediation process itself, but can be used also after treatment (Dostalek [2011](#page-8-0)). Murray and Kidby ([1975](#page-8-0)) showed that the mercury ions are absorbed on the nonprotein part of the cell walls of yeast, which consists of β-(1-3)-glucans chains which bonded by β-(1-6)-glucan (Manners et al. [1973\)](#page-8-0). To separate effectual metal sorbent from the yeast, various combinations of physical and chemical methods were applied. These include vacuum and freezedrying, boiling or heat, autoclaving, mechanical disruption, and treatment with organic and inorganic reagents (Wang [2002;](#page-9-0) Wang and Chen [2006\)](#page-9-0). A sorbent that effectively accumulates heavy metal ions Cd, Cu, and Ag was prepared by alkali treatment of yeast followed by rinsing in water and drying with organic solvents. The sorbent consists of tiny particles, which were entrapped in silica to form packing of a sorption column (Szilva et al. [1998](#page-9-0)).

The objective of this study was to improve sensitivity of detection of Hg^{2+} with bioluminescent bioreporter E. coli ARL1 via preconcentration of mercury on yeast sorbent prepared by alkali treatment followed by rinsing in water and spray drying. It is known that this sorbent effectively accumulates heavy metal ions Cd, Cu, and Ag (Patzak et al. [1997](#page-8-0); Szilva et al. [1998](#page-9-0)). We also demonstrated that Hg^{2+} is firmly adsorbed by the sorbent and that mercury ions are not washed out by water, phosphate-buffered saline (PBS), or salty solutions. We developed detection method based on induction of bioluminescence by mercury adsorbed on this sorbent immersed in a medium with tryptone.

Experimental

Microorganisms

Escherichia coli (EC100) ARL1 (Dahl et al. [2011\)](#page-8-0) was kindly donated from the collection of microorganisms of CEB University of Tennessee, Knoxville, USA.

Saccharomyces pastorianus strain RIBM 95 from the Culture Collection of Research Institute of Brewing and Malting (RIBM) stored in Research Institute of Malting and Brewing in Prague was used for the preparation of cell wall envelopes and then sorbent.

Materials and solutions

The following materials were used: sodium and mercury chlorides, ethylenediaminetetraacetic acid disodium salt dihydrate, EDTA (Lach-Ner, Czech Republic), phosphates, (Penta, Czech Republic), tryptone (Oxoid, England), yeast extract, kanamycin, and D-glucose (Sigma-Aldrich, USA).

The phosphate buffer (PB) (pH 7.4) contained KH_2PO_4 (1.7 mmol/L) , Na₂HPO₄ (5.2 mmol/L), and PBS (pH 7.4) was prepared by supplementing PB with NaCl (0.15 mol/L). D-Glucose stock solution (2 mol/L in dH_2O) was sterilized by filtration through a syringe filter (pore size $0.22 \mu m$; Millipore, France). Luria–Bertani media (LB) contained tryptone (10 g/L), yeast extract (5 g/L), NaCl (10 g/L), pH 7.2 (Sambrook et al. [1989\)](#page-8-0). The LB+kan medium was prepared by the addition of a stock kanamycin solution (10 g/L) to the final concentration of 50 mg/L. The tryptone solution (20 g/L) was sterilized in an autoclave.

Soil solution (LP3586) and stream water (LP488) containing naturally low ambient concentrations of mercury were sampled at small forest catchment Lesnípotok near Prague, Czech Republic. Details on site description can be found in Navrátil et al. ([2011,](#page-8-0) [2014\)](#page-8-0), and for chemical analysis of studied water samples, see supplement (Table S1).

Preparation of S. pastorianus cell wall envelopes

The preparation was based on the method described by Patzak et al. ([1997](#page-8-0)). Spent brewer's yeast (S. pastorianus) slurry (solid content 15 $\frac{v_{0}w}{v}$ was collected at the end of beer primary fermentation and store at 2 °C. The yeast slurry was exposed to cell autolysis for 24 h at 50 °C, and subsequently, the solid fraction was separated from the yeast extract by centrifugation (Saksinchai et al. [2001\)](#page-8-0). Further treatment was carried out on

the solid fraction (20 g/L) at 80 °C using 1 M NaOH for 1 h. Then, the mixture was cooled, centrifuged, and washed with water and spray-dried at 50 °C. The obtained yeast envelopes (microparticles) rich in β-glucan were used for subsequent work.

Sorbent preparation

Yeast envelopes (0.1 g) were resuspended in a dH₂O (10 mL) , and after sedimentation (10 min), the supernatant was decanted. This operation, sedimentation and decantation, was repeated three times. Finally, sediment, which contained globular cell wall aggregates with mean dimensions \sim 20 μ m, was resuspended in dH_2O (5 mL) to form the sorbent suspension.

Cultivation of E. coli ARL1

The overnight culture was inoculated into a fresh LB medium with kanamycin and incubated at 37 °C, 200 rpm to OD_{600} approximately $0.6 \approx 1 \times 10^8$ colony-forming units per milliliter) (measured by UV-VIS spectrophotometer HP8452A, Hewlett-Packard, USA). The bacterial cells were centrifuged for 10 min at 2600 rpm, and a pellet was resuspended in PBS with glucose (40 mmol/L) and tryptone (10 g/L) to a cell concentration of 2×10^8 colony-forming units per milliliter. This suspension was used for bioluminescence induction in 96-well microplates.

Characterization of the sorbents

Specific surface (BET)

Nitrogen adsorption-desorption (Digisorb 2600 V4.02, BET) was used to determine the specific surface area of the sorbent samples.

Optical microscopy

Carbolfuschin (5 μ L of 10 g/L) was added to sorbent suspension and observed in Bürker chamber with optical microscope Carl Zeiss Primo Star.

SEM

The samples of yeast envelopes and sorbents were coated with gold by EMITECH Sputter Coater K500X for 2 min under sputtering current 50 mA. The gold-coated samples (thickness of gold layer was ~30 nm) were scanned by scanning electron microscope (SEM) Tescan Indusem.

Preparation of the sorbent with E. coli ARL1 for SEM

The wet sorbent (0.5 mg dry weight) was mixed with suspension of E. coli ARL1 (250 μL) containing E. coli ARL1 (10⁸) colony-forming unit (cfu)/mL), glucose (20 mM), PBS buffer, tryptone (5 g/L), and incubated (1.5 h), 37 °C. After incubation, sorbent with bacterial cells were washed with dH_2O (2 mL), centrifuged 1000 rpm, and dried under ambient condition.

EDX analysis

Elemental analyses were made with Quantax 200 and XFlash detector 5010 instruments from Bruker for energy-dispersive X-ray spectroscopy (EDX) mounted on SEM. Aqueous sorbent suspension (100 μL) was placed into aluminum cylindrical holder (\varnothing 6 mm, height 2 mm) and dried under ambient conditions. Data were collected from square area $(2\times2$ mm) for 5 min at accelerating voltage 30 kV.

The sensitivity of the EDX analyses is about 0.02 %. With the aim to demonstrate mercury adsorption and desorption, the samples for EDX were prepared to be mercury saturated.

Preparation of a sorbent suspension for EDX analysis

- 1. Sorbent was prepared as described above (see "Sorbent" preparation") without additional treatment.
- 2. Sorbent treated with EDTA: EDTA solution (400 μL, 0.5 mol/L) was added to the sorbent suspension (1.5 mL) and gently shaking for 2 min at ambient temperature. After incubation, samples were washed three times with dH_2O (1 mL) and centrifuged.
- 3. Sorbent with adsorbed Hg^{2+} : Sorbent suspension (50 mg) dry weight of the sorbent) was centrifuged. The sedimented sorbent was resuspended in 2 mL of solution of HgCl₂ (5 g/L). After incubation (2 min of gently shaking), the sorbents with Hg^{2+} were washed with dH_2O (1 mL) and centrifuged. The washing and centrifugation was repeated three, five, or ten times.
- 4. Sorbent with adsorbed Hg^{2+} eluted with tryptone: The sorbent with adsorbed Hg^{2+} (50 mg dry weight) was resuspended in tryptone solution (2 mL, 20 g/L) and gently shaken for 2 min at ambient temperature. The tryptoneeluted sorbents were washed with water three times.

Determination of mercury concentrations with CV-AAS and CV-AFS

Samples (prepared for EDX) were dissolved in 25% HNO₃ (5 mL) , and diluted in dH₂O (to 50 mL). Mercury concentration in solid samples as well as in liquid samples with Hg concentration higher than 50 ng/L were quantified using Scheme 1 Sequence of procedures and characterization of the yeast cell sorbents and bioluminescence inductions

AMA254 Advanced Mercury Analyzer (Altec, Czech Republic) in standard conditions recommended by the producer (100 μL of samples, 1-mL sample holder, 120 s evaporation, 150-s degradation of samples, 45-s cooling and data collection). In diluted liquid samples, mercury was analyzed by AFS mercury vapor fluorescence system Millennium Merlin (PS Analytical, England). The instrument was operated in the Galahad mode, using standard conditions according to the application note PSA AFS HG 1631 compatible with US EPA 1631 methodology (US EPA [2002\)](#page-9-0).

Bioluminescence of E. coli ARL1

Elution tests

Adsorption of Hg^{2+} To demonstrate mercury elution from the sorbent, mercury was adsorbed from solution $HgCl₂$ (10 μ g/L), which induced bioluminescence without preconcentration. The sorbent suspensions (0.5 mL (10 mg dry weight)) were aliquoted into individual 2-mL Eppendorf microcentrifuge tubes. In each tube, $HgCl₂$ solution (1 mL, 10 μg/L) were added and after mixing by vortex samples were left on the table (10 min) and centrifuged at 9000 rpm (10 min) (Universal 32R, rotor 1689-A, Hettich, Germany). After centrifugation, supernatant was filtered through 0.45-μm syringe filter into another tube. A sediment was washed three times with dH₂O (1 mL) and centrifuged. This sediment, sorbent with Hg^{2+} , containing 0.738 ng $_{Hg}/mg_{\text{softent}}$ (calculated under condition of full sorption), was further used in elution tests.

Elution of Hg^{2+} Elution was carried out using PBS, 0.1, 0.5, and 1 M NaCl (0.5 mL) by bath method. The sorbent with Hg^{2+} (10 mg dry weight) was vortexed with an eluent and incubated 10 min at ambient temperature. After centrifugation supernatant (eluate) was filtrated trough syringe filter (0.45 μ m) and placed to a new tube. Filtered eluate (50 μ L)

Fig. 1 SEM cell envelopes before (a, b) and after (c, d) separation by sedimentation in dH₂O and after EDTA treatment (e, f) . b, d, f Enlarged images of the particles

was used for bioluminescence induction of E. coli ARL1. A sediment was washed three times with dH_2O (1 mL) and finally resuspended in a $dH₂O$ (0.5 mL). The suspension of the sediment (50 μ L) was placed into a well, and bioluminescence was induced.

Induction of bioluminescence In a 96-well microplate, $10\times$ PBS (12.5 μL) were pipetted and cell suspension was prepared for induction (125 μ L) to make a cell concentration of 1×10^8 cfu/mL. Finally, an eluent (50 μ L) and dH₂O (62.5 μ L) or suspension of sorbent with adsorbed Hg $(100 \mu L)$ and $dH₂O$ (12.5 μ L) were added.

Bioluminescence was measured 15 h using a Spectrostar Omega (BMG Labtech, Germany) at 37 °C (from the plate

Fig. 2 Water suspension of the sorbent stained with carbolfuschin

bottom, 4 s every 7 min). All experiments were performed in triplicate.

Tests of detection assay

Preparation of samples Sorbent suspension (10 mg dry weight of the sorbent) was added to spiked water (15 mL, 10 ng/L of HgCl₂) and to field samples (15 mL, LP 488 or LP 35 86, filtered through 0.45-μm syringe filter); after that, the sample was incubated for 5 min with gentle shaking. After incubation, samples were centrifuged (9000 rpm) and sedimented sorbent was resuspended in dH_2O (100 μ L). Distilled and tap water (Hg=17.0 \pm 0.3 ng/L, determined by AFS) was spiked with 20 and 100 ng/L $HgCl₂$.

This sorbent suspension (100 μ L) was placed into a 96well microplate, and $10 \times$ PBS (12.5 μ L) and dH₂O (12.5 μ L) were added. Finally, each well was supplemented with the cell suspension cultivated for induction (125 μL) and bioluminescence was measured as described above.

Results

Sequence of procedures and characterization of the yeast cell sorbents and bioluminescence inductions is shown in Scheme [1](#page-3-0).

Fig. 3 Contents of Hg^{2+} determined by AAS and EDX. Mercury was adsorbed on the sorbent (50 mg) from 2 mL of solution HgCl₂ (5 g/L)

The sorbent

Dry agglomerates of cell wall envelopes were irregular spheres (external diameter of $10-30 \mu m$) with holes and sags (Fig. [1a, b](#page-4-0)). This agglomerate shape was made by nebulization. In contrast to that cell wall envelopes dried with organic solvents formed light-brown powder having particle dimension \sim 2–5 μ m (see Fig. 1 in Szilva et al. [1998\)](#page-9-0). In water, the agglomerates swelled and gradually settled (Fig. [2\)](#page-4-0). This allowed separation of aggregates having regular spherical shape (Figs. [1c, d](#page-4-0) and [2\)](#page-4-0) via sedimentation in water. After drying this fraction, consisting from aggregates diameters from 20 to 30 μm, was employed as a sorbent. Specific surface (BET) of the cell envelopes was 0.4682 m^2/g and the sorbent $0.3701 \text{ m}^2/\text{g}$. After treatment with EDTA, appearance of particles was changed (Fig. [1e, f](#page-4-0)) and specific surface of the

Fig. 4 EDX analysis: a the sorbent before (dash-dot line) and after (solid line) EDTA treatment; b EDTA-treated sorbent before (solid line) and after (dashed line) sorption Hg^{2+} ; c the sorbent before (solid line) and after (*dashed line*) sorption Hg²; **d** the sorbent with Hg²⁺ before (*solid* line) and after (dashed line) three times tryptone washing

Fig. 5 Integrated bioluminescence induced with sorbent, sorbent after elution, and eluates. Sorbent with Hg (on sorbent (10 mg dry weight) was adsorbed HgCl₂ (1 mL, 10 μ g/L). This sorbent was eluated with water, PBS, and NaCl (1 mL)

sorbent decreased to $0.2514 \text{ m}^2/\text{g}$. The sorption capacity Hg²⁺of the sorbent was 17.7 \pm 0.1 μmol/g (calculated from CV-AAS measurements), and in case of the sorbent without calcium, this capacity dropped down to 2.88 μmol/g. Adsorbed mercury ions were not removed from the sorbent (or sorbent without calcium) by repeated washing with water (Figs. [3](#page-5-0) and [4a](#page-5-0)).

Induction of bioluminescence

With aim to find conditions for induction of bioluminescence of E. coli ARL1 with concentrations of mercury ions that are allowable in ground and drinking water, we tested bioluminescence inductions of both the sorbent with mercury and eluates.

Eluates, solutions of salts, induced ten times lower bioluminescence as compared to rinsed sorbents (Fig. 5). EDX analysis of sorbent surfaces revealed that the contents of mercury ions (for other elements, see supplement, Table S2) on sorbent decreased by elution with PBS to 54 %, by elution with salty water (NaCl $0.5-2$ M) to $31-34$ % and with

Fig. 7 Time record of bioluminescence of E. coli ARL1 induced with aqueous solutions of HgCl₂ with concentrations from 75 to 2500 ng/L and the sorbent (1 mg) with Hg^{2+} adsorbed from 15 mL of $HgCl_2$ solution (20 ng/L)

tryptone to 17 % (Figs. [3](#page-5-0) and [4d](#page-5-0)). Murray and Kidby [\(1975](#page-8-0)) had demonstrated that mercury ions are bound by complexation with polyglycans. Complexation of Hg^{$2+$} with tryptone amino groups is stronger as compared to complexation with hydroxy groups of polyglycans of cell wall envelopes (Sillén et al. [1971\)](#page-8-0). Under conditions of bioluminescence induction, bacteria E. coli ARL1 covered sorbent to form continuous cell layer (Fig. 6). The bioluminescence maximum with sorbent was reached after 1.5 h in contrast to 30 min needed for induction with mercury ions in solutions (Fig. 7). Nevertheless, this bioluminescence maximum, with sorbent, was higher in comparison to that induced by the same Hg^{2+} concentration in solution (compare lines of the sorbent and solution with 1.25 μg/L, Fig. 7). Prolonged time when appeared bioluminescence maximum related with an increasing mercury concentration as we observed in solutions (see supplement, Figs. S1 and S2). Sorbent with adsorbed Hg^{2+} was covering with cells at the same time as tryptone eluated mercury from this sorbent (Fig. 6). This way concentration of mercury increased in close proximity to the cell layer adhering to the sorbent.

We apply this preconcentration method for detection of mercury in two samples of field water (see supplement,

Fig. 6 E. coli ARL1 on the sorbent with adsorbed Hg^{2+} under condition of bioluminescence induction

Fig. 8 Integrated bioluminescence of E. coli ARL1 induced in medium with tryptone by underground (LP-498 and LP3586) and spiked water (10 ng_{HgCl2}/L) without preconcentration and after preconcentrations (from 15 mL) on the sorbent (10 mg dry weight)

Table S1). Without preconcentration, stream and soil water with Hg concentrations of 1.9 and 10.9 ng/L, respectively, did not induce higher bioluminescence than the distilled water. After preconcentration, bioluminescence was not induced with stream water as well and the maximum induced with soil water was about 50 % lower as compared to the bioluminescence induced with the same amount of mercury in distilled water (Fig. 8). Both real samples contained, except mercury, metals and DOC. DOC interacts very strongly with mercury, affecting its speciation, solubility, mobility, and toxicity in the aquatic environment (Ravichandran [2004](#page-8-0)). In previous work in our laboratory, we observed a negligible bioluminescence of E. coli ARL1 in stream water (LP 3586) even if content of HgCl₂ was increased up to 800 ng/L. This bioluminescence suppression was ascribed to DOC on the basis of experiments that demonstrated vanishing of E. coli ARL1 bioluminescence in $HgCl₂$ spiked water after addition of humic acids, which is considered as the main component of DOC (Bartošková

[2013\)](#page-8-0). In stream water, having low concentration of Hg and high content of DOC, all mercury ions were bound to DOC, which prevented preconcentration by adsorption on the sorbent. Samples of soil and stream water contained iron and manganese in concentrations that interfere with induction of bioluminescence of mercury (see supplement, Fig. S3). Therefore, in such samples, the detection of mercury by the preconcentration method is not reliable.

Though in tap water, CV-AAS analysis revealed mercury $0.084\pm5\times10^{-6}$ nmol/L $(16.8\pm1.0\times10^{-3}$ ng/L) bioluminescence maxima induced (after preconcetration) with tap water and distilled water were identical (Fig. 9a). Nevertheless, integrated bioluminescence of tap water was within experimental error, slightly higher (Fig. 9b). We spiked both tap and distilled water with 20 ng/L $HgCl₂$. The bioluminescence, both maximum and integrated, of spiked tap water was higher, and using the method of standard addition (Marques and Esteves da Silva [2008](#page-8-0)), we calculated the tap water mercury 0.127 ± 0.09 nmol/L (25.46 \pm 18.04 ng/L).

Discussion

Cell wall envelopes are sorbent that adsorbed mercury ions from water with sorption capacity 17.7 ± 4.4 μ mol/ g. Extraction of calcium ions with EDTA decreased specific surface area and sorption capacity. This effect is probably a result of a modification of sorbent surface structure (compare Fig. [1c, d and e](#page-4-0), f) as was noticed by De Nobel et al. [\(1989\)](#page-8-0). In presence of EDTA, Bishnoi and Garima ([2005\)](#page-8-0) also had observed reduction of biosorption studying bioremediation with fungus.

The sorbent was used for increasing sensitivity of detection of mercury ions with bioluminescent bioreporters E. coli ARL1. The novel method of detection is based on induction of bioluminescence by mercury adsorbed on the sorbent immersed in a medium with tryptone. A limit of detection of this

Fig. 9 Bioluminescence of E. coli ARL1 induced in medium with tryptone by tap water and spiked water (10 and 100 ng_{HgCl2}/L) after preconcentrations (from 15 mL) on the sorbent (10 mg dry weight); maxima (a) and integrated (b)

method was 20 ng/L in contrast to LOD 500 ng/L, which was reached without the sorbent (Solovyev et al. [2015](#page-9-0)).

The method with preconcentration was applied for mercury detection in three field water samples. In these samples, mercury concentrations were below the EPA limits for drinking water: in USA, 2 μg/L (Environmental Protection Agency 2009), and in EU, 1 μg/L (Council Directive 1998).

Stream and soil water, except mercury, contained metals along with DOC and humic acids. Iron and manganese, concentrations of tenths micrograms per liter, induced E. coli ARL1 bioluminescence. On the contrary, DOC and humic acids suppressed bioluminescence induction because they both bind mercury. This bounded mercury, biologically unavailable for bioreporter E. coli ARL1, was neither released by tryptone nor concentrated on cell walls. Soil, stream, or service water commonly contained both components, which inversely interfere with the induction of bioluminescence by mercury. In such samples a detection of bioavailable mercury based on induction of bioluminescence of E. coli ARL1 after mercury preconcentration is not reliable.

In tap water, in which contents of other metals and organic compounds used to be so low that does not interfere mercuryinduced bioluminescence of E. coli ARL1, we demonstrated a mercury detection with the method of standard addition.

In conclusion, we studied a detections method based on induction of bioluminescence by mercury adsorbed on this sorbent that make possible to detect mercury in tap water in concentrations below the EPA limits for drinking water: in USA, 2 μg/L (Environmental Protection Agency 2009), and in EU, $1 \mu g/L$.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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