Rapid measurement of the waterborne pathogen Pseudomonas aeruginosa in different spiked water sources using electrochemical sensing

Towards on-site applications

Alatraktchi, Fatima AlZahraa

Published in:
Measurement

DOI:
10.1016/j.measurement.2022.111124

Publication date:
2022

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Alatraktchi, F. A. (2022). Rapid measurement of the waterborne pathogen *Pseudomonas aeruginosa* in different spiked water sources using electrochemical sensing: Towards on-site applications. *Measurement*, *195*, [111124]. https://doi.org/10.1016/j.measurement.2022.111124

Measurement 195 (2022) 111124

Contents lists available at ScienceDirect

Measurement

journal homepage: www.elsevier.com/locate/measurement

Rapid measurement of the waterborne pathogen *Pseudomonas aeruginosa* in different spiked water sources using electrochemical sensing: Towards on-site applications

Fatima AlZahra'a Alatraktchi

Department of Science and Environment, Roskilde University, Universitetsvej 1, 4000 Roskilde, Denmark

ARTICLE INFO

Keywords:
Pseudomonas aeruginosa
Pyocyanin
Electrochemical sensing
Voltammetry
Water sources
Pathogen monitoring

ABSTRACT

Pseudomonas aeruginosa is a waterborne ubiquitous opportunistic pathogen that can be fatal for immunocompromised patients. Here, we report the use of paper-based screen-printed sensors for the electrochemical detection of *P. aeruginosa* in water sources in only 14 s. *P. aeruginosa* is identifiable by its unique biomarker pyocyanin, which is redox-active and therefore suitable for direct electrochemical detection without sample pretreatment. The detection method was applied to detect pyocyanin directly in spiked lake water, tap water, sea water and groundwater. The electrochemical response was linearly proportional to the pyocyanin concentration with R^2 values between 0.9918 and 0.9991. Additionally, we demonstrated the direct detection of *P. aeruginosa* in water in concentrations ranging from 5 to 50 colonies/mL. The results are a step towards utilizing simple electrochemical sensing to rapidly determine if water sources are infected with *P. aeruginosa* prior to bathing, consumption or other use.

1. Introduction

Waterborne diseases pose a major risk to immunocompromised patients [1]. Waterborne pathogens are related to environmental deterioration, making the water a source of disease transmission [2]. Pathogens can be transmitted to humans and animals through drinking water, bathing, washing or by eating food exposed to contaminated water. Although a significant proportion of fatal water-related pathogenesis are caused by deadly pathogens as *Vibrio cholera* and *Salmonella typhimurium*, opportunistic pathogens present imperative challenges to the public health [3-6].

Pseudomonas aeruginosa is a waterborne opportunistic pathogen commonly found in lakes, sea water, swimming pools and even in drinking water. *P. aeruginosa* is a gram-negative bacterium that has the ability colonize even distilled water with limited available nutrients. Additionally, *P. aeruginosa* has a characteristic ability to form biofilms which are especially problematic in showerheads, faucets, whirlpools and other plumbing fixtures [7,8]. The most elevated risk of biological pollution of water is assigned to *P. aeruginosa* contaminating water stored in tanks [9,10], water distributed in pipeline systems and household water filters. *P. aeruginosa* have a high resistance to disinfectants as chlorine and alcohols and can grow at temperatures up to 42 °C [11].

Numerous outbreaks with *P. aeruginosa* infections caused by consumption of contaminated water have been reported in literature. Infection sources include drinking water supply, contaminated batches of bottled water or even aerosol exposition of contaminated water from water in dental chair units. In specific outbreak cases, hospital water systems have also been reported to be positive for *P. aeruginosa* in majority of tested samples[4,12,13].

P. aeruginosa infections upon drinking contaminated water causes diarrhea, vomiting with following severe dehydration. Especially at risk are immunocompromised patients that are in propensity of developing rapidly fatal *P. aeruginosa* infections include patients with burns or open wounds, neutropenia following neoplasms, cystic fibrosis and hospitalized patients in intensive or special care units [14]. Contact with *P. aeruginosa* contaminated water through consumption of water, inhalation of water droplets or skin contact may lead to a range of infections that in severe cases are impossible to treat due to the multi-drug-resistance that *P. aeruginosa* can develop [15,16].

In the European Union, several water directives demand mandatory measurements of *P. aeruginosa* in drinking water sources as a part of the water control programs [17,18]. There is zero-tolerance for *P. aeruginosa* in drinking water resources. However, the detection of *P. aeruginosa* in

E-mail address: alzahraa@ruc.dk.

https://doi.org/10.1016/j.measurement.2022.111124
Received 16 November 2021; Received in revised form 11 February 2022; Accepted 30 March 2022
Available online 1 April 2022

water sources is troublesome due to time-consuming methods requiring laboratory heavy procedures and expensive labor costs [19]. The water samples need to be collected manually from the respective fields and transported to laboratories for culturing or polymerase chain reaction (PCR) analysis. This limits frequent control and only provides snapshots of the contamination situation. By the time answers are collected from the laboratories, human exposure may already have occurred. Thus, there is a need for rigorous facility maintenance and monitoring using tools that allow instant, on-site detection of *P. aeruginosa* in e.g. sea water in bathing seasons, hot tubs and saunas before use and in drinking water sources [15,20].

A distinct feature of *P. aeruginosa* is that it produces the redox-active toxin pyocyanin which is possible to detect electrochemically [21]. Electrochemical detection of compounds as a high-throughput analytical tool is a rising area that is finding its way to field applications. The electrochemical sensing field covers detection of analytes ranging from biological products to chemical substances [22-24]. Several studies have established the possibility of electrochemical detection of chemical contaminants directly in water, such as pesticides and toxic heavy elements [25-30]. Although electrochemical detection of pyocyanin in culture media for microbiology or clinical research has repeatedly been published [21], to the authors' knowledge, electrochemical detection of pyocyanin as an identification biomarker for *P. aeruginosa* directly in water has never been reported. Although pyocyanin has earlier been demonstrated to be measured in growth media and patient samples, the direct application in water is novel and may open possibilities for real on-site monitoring of *P. aeruginosa* which potentially could advance water safety measures.

Here, we present simple electrochemical detection of pyocyanin in various water resources, using screen-printed paper sensors. The detection can be conducted without any pretreatment of the water samples and results can be obtained in 14 s. The proof-of-concept results suggest that low-cost paper-based sensors can potentially be used for on-site, immediate analysis of *P. aeruginosa* in water.

2. Materials and methods

2.1. Instrumentation and reagents

Sensor characterization was conducted in 10 mM ferri/ferrocyanide in phosphate buffer saline (PBS) by mixing Potassium hexacyanoferrate (II) triphydrate (P23289-110G, Sigma-Aldrich, Denmark) and Potassium hexacyanoferrate(III) (31253-250G, Sigma-Aldrich, Denmark). Graphene ink (C2171023D1, Sun Chemical Ltd, UK) was used for screen-printing through a screen with a mesh of 14 μm of film emulsion with a mesh thickness of 80 ± 2 μm on hard paper (C2549-100, Avery Zweckform).Tap water was collected from the tap of the laboratory at Roskilde University, while lake water was collected from the local lake at Roskilde University (location: 55°39′05.8″N 12°08′03.6″E). Ground water was sampled from a local water work (Gevninge Vandværk, Denmark) immediately after pumping from the well and used with no further treatment. Sea water was collected from Greve beach, Denmark (location: 55°35′01.7″N 12°19′26.7″E). The collected waters were tested for *P. aeruginosa* growth by streaking samples on *P. aeruginosa* selective plates. No growth was observed. Pyocyanin was extracted from a PAO1 overnight culture according to El-Shouny et al [31]. All electrochemical measurements were conducted versus the quasi-reference electrode using a potentiostat (Emstat Blue, Palmsens, Netherlands). The electrochemical data analyses were performed using PStrace V5.8 software (PalmSens, Houten, The Netherlands). A fresh sensor was used for each single measurement.

2.2. Sensor fabrication and characterization

The fabrication process of the paper-based sensors was based on manual screen-printing by forcing the graphite ink through a negative pattern of the sensors. As the blade was moved across the screen (mesh size of 63 μm), the graphite ink was forced down on the paper, creating a sheet of sensors similar to the process depicted in Alatraktchi et al. [32]. The sensors consisted of three-electrode configuration design counting a working electrode (WE), a counter electrode (CE) and a quasi-reference electrode (RE) all made of graphite. After screen-printing, the sheet is cured at 80 °C for 30 min and the individual sensors are cut out with a scissor. Characterization was conducted in 2.5 mM ferri/ferro-cyanide in PBS. The characterization was obtained by performing cyclic voltammograms from −1.0 V to + 1.0 V, using a potential step of 0.01 V and scan rates ranging from 0.03 to 0.25 V/s.

2.3. Identification of pyocyanin signal in water

Cyclic voltammograms of tap water and 5 μM pyocyanin in tap water was performed from –0.75 V to −0.25 V, using a potential step of 0.01 V and a scan rate of 0.08 V/s. Pyocyanin concentration of 200 μM was spiked in tap water and in PBS, respectively, and measured with square wave voltammetry (SWV) from −0.90 V to 1.0 V at a step potential of 0.005 V and a frequency of 10 Hz to characterize the peak positions and effect of the electrolyte on signal strength.

2.4. Quantification of pyocyanin

Square wave voltammetry (SWV) was used to quantify pyocyanin at the characteristic peak potential around −0.6 V using a scan range from −0.90 V to −0.3 V at a step potential of 0.005 V and a frequency of 10 Hz according to slightly modified parameters from a previous report for paper-based sensors [32], resulting in a measurement time of 14 s. Dilution series of pyocyanin from 1.56 to 100 μM were prepared in PBS, tap water, ground water, lake water and sea water, respectively, and measured in triplicates by SWV. All measurements have been conducted against the quasi-reference electrode.

2.5. Determining temperature, pH and electrolyte dependency

A fixed pyocyanin concentration of 20 μM diluted in tap water was measured and divided in five separate vials and stored for five hours at 4, 25, 30, 37 and 42 °C before measurements. The temperatures of the samples were controlled prior to SWV measurements in triplicates. Statistical significance between the data sets were analyzed by one-way ANOVA followed by a *t*-test with a probability value > 0.05.

The pH of the water sources and PBS was determined using pH strips (85410.601P, Paper dosatest, Prolab, VWR). To test the signal dependency, a fixed pyocyanin concentration of 145 μM in tap water was prepared in several vials and each vial was pH adjusted to a pH range between 4.0 and 9.5. All measurements were performed at room temperature. The samples were electrochemically measured in triplicates.

The electrolyte dependency mas determined by spiking 20 μM pyocyanin in tap water with 0, 0.1, 0.5 and 1.0 M KCl. Likewise, PBS was diluted in milli-Q water and a constant pyocyanin concentration of 35 μM was spiked in the dilution series from 6.25 to 100 % PBS. All samples were electrochemically measured in triplicates.

2.6. Test of interference from another microorganism

Overnight cultures of *Escherichia coli* and PAO1 were prepared in accordance with Mojsoska et al. 2021 [33]. Three different vials with lake water were spiked with equal volumes of (1) *E. coli*, (2) E. coli and PAO1 and (3) PAO1, respectively. Three electrochemical measurements were taken from each vial using the aforementioned SWV parameters.

2.7. Detection of P. Aeruginosa in water

The laboratory *P. aeruginosa* reference strain, PAO1, was streaked on lysogeny broth plates for 48 h until they showed their typical green–blue

pigment. Single colonies of 5–50 were suspended in vials of 1 mL lake water. The colonies were manually counted and transferred from agar plates to the vials with water using sterile loops. Subsequently, the paper-based sensors were used to quantify the pyocyanin content in the respective samples using SWV. Measurements were done in triplicates.

3. Results and discussion

3.1. Characterization of paper-based sensors shows quasi-reversible behavior

The reliability of the paper-based graphene sensor was evaluated by CV measurements of ferri/ferrocyanide using varying scan rates (Fig. 1**a**). The current peak heights were extracted from the voltammograms and plotted against the square root of the scan rate, showing a linear relation with an R2 fit of 0.992 and 0.985 (Fig. 1**b**). The numeric peak current ratios between the oxidation currents I_{ox} and the reduction currents I_{re} were observed to be deviating from one with approximately 13 %. Additionally, the peak potentials increase with increasing scan rates. As the sensor slightly deviate from the reversibility criteria, the sensor is considered quasi-reversible which is sufficient for reliable analytical quantification applications.

3.2. Electrochemical pyocyanin signal obtained directly in water

The electrochemical response of pyocyanin in tap water was investigated by CV in the negative potential window as reported by previous studies [32]. The CV of pyocyanin spiked in tap water was measured against the background (Fig. 2**a**). The voltammograms depicts quasi-reversible response with an oxidation peak at −0.51 V and a reduction peak at −0.58 V. Within this potential window, the observed redox couple is exclusively due to the conversion between the monomeric forms (I) and (II) of pyocyanin [34-36]. As no background peaks are observed from the blank tap water, it is concluded that it is possible to obtain a unique pyocyanin signal in water without the addition of any electrolyte.

Comparing the SWVs of pyocyanin spiked in PBS and tap water, we see a shift in the peak potential at which the monomeric redox reaction is appearing (Fig. 2**b**, peak III), which is due to the different salt content of PBS and tap water. The peak current obtained from measuring pyocyanin in PBS is approximately double as high as pyocyanin measured in tap water. This is attributed to the higher salt content in PBS compared to tap water which makes PBS a stronger electrolyte to carry the current. Next to the pyocyanin peak, a smaller secondary peak can be observed in the pyocyanin in tap water measurement. This peak is likely a result of measuring the residue 5-methylphenazine-1-carboxylic acid, a precursor to pyocyanin synthesis [37] (Fig. 2**b**, peak IV). This peak is also occurring in the spiked PBS measurements (**Fig. S1**), however; due to the larger peak current of pyocyanin and the shift of the peak potential, it is not visible. Further, a phenolic oxidation of pyocyanin

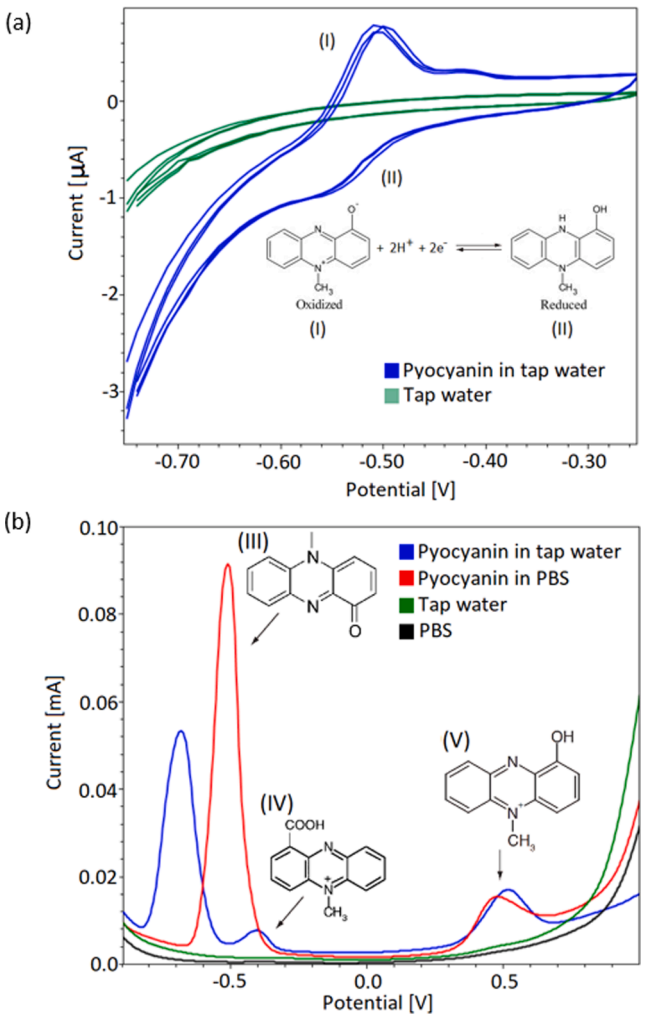

Fig. 2. Electrochemical fingerprint of pyocyanin in water. (a) Cyclic voltammograms of 5 µM pyocyanin in tap water measured with the paper-based sensors. (b) Square wave voltammograms of tap water and PBS spiked with 200 µM pyocyanin vs the backgrounds.

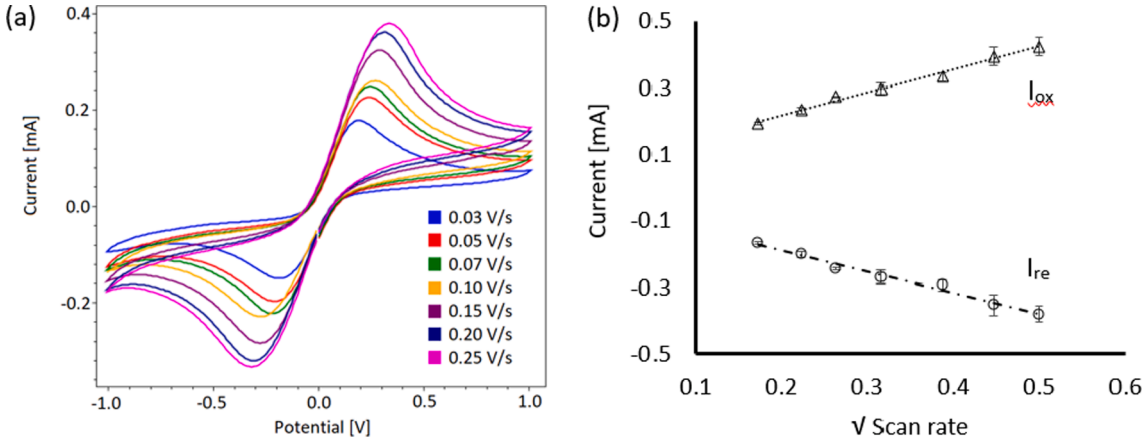

Fig. 1. Electrochemical characterization of the paper-sensor. (a) Cyclic voltammograms with increasing scan rates measuring 2.5 mM ferri/ferrocyanide. (b) The extracted peak current values as function of the square root of the scan rates.

occurs at + 0.5 V [34,38] (Fig. 2**b**, peak V). This peak is also responsive to concentration changes as seen in **Fig. S2**. However, as the peak at the negative potential is significantly higher than at + 0.5 V and no background peak is observed, the potential scan is limited to the negative potential window for the further analytical measurements.

3.3. Electrochemical pyocyanin signal intensity is concentration dependent

Square wave voltammograms of spiked PBS shows increasing peak heights with increasing pyocyanin concentration (Fig. 3**a**). The non-faradaic current was filtered out from the peak currents by subtracting the blank water background measurements. The faradaic peak-heights for each set of measurements in the four different water types and PBS were then extracted and plotted as function of the corresponding concentrations as seen in Fig. 3**b**. The linear range between 1.56 and 100 μM for each of the different water types has fitted lines with R2 values above 0.99. There is a difference in the conductivity of the water sources observed by the variance in the slopes of the standard curves. The slope of the linear fit of a standard curve is also a measure of the sensitivity. The limit of detection (LoD) was calculated as 3 times the standard deviation of the lowest concentration measured divided by the sensitivity. The sensitivity and the LoD were calculated for each water type as listed in Table 1. The LOD was not consistent across the different types of water and PBS. The average LOD of the waters was 0.63 ± 0.24 μM. However, there is no statistical difference between the values. As the extracted peak currents are directly proportional to the diffusion coefficient of pyocyanin, the water matrix will significantly affect sensitivity.

Table 1
Pyocyanin sensitivity and limit of detection (LoD) for each water type.

Water type	Sensitivity [μA/μM]	LoD [μM]
PBS	0.607	0.425
Lake water	0.338	0.308
Sea water	0.307	0.859
Tap water	0.259	0.492
Ground water	0.236	0.862

Thus, it is observed that the PBS has the highest sensitivity where the salt content is high while groundwater has the lowest sensitivity probably due to the lowest salt content of the tested waters sources. While the sensitivity of measuring pyocyanin in water is lower than detecting pyocyanin in PBS, the overall results show that pyocyanin can be detected directly in water samples with no sample pretreatment or need of electrolyte addition.

To test if temperature has an effect on the intensity of the signal, spiked tap water was measured at different temperatures from 4 to 42 °C (Fig. 3**c**). There was no statistical difference between the measured signals, suggesting that electrochemical measurements of *P. aeruginosa* can be conducted on-site, e.g., in lakes or in hot tubs, independent of the temperature.

The pH of the collected water sources, tap-, sea-, lake- and groundwater was 7.5 while the pH of PBS was 7.0. The influence of electrolyte pH on the peak potential and peak current of the pyocyanin signal was investigated by SWV measurements in the pH range of 4.0 to 9.5. Fig. 4**a** illustrates that the peak potential response shifted to more negative values when increasing the pH from 4.0 to 5.5 and at higher pH values it

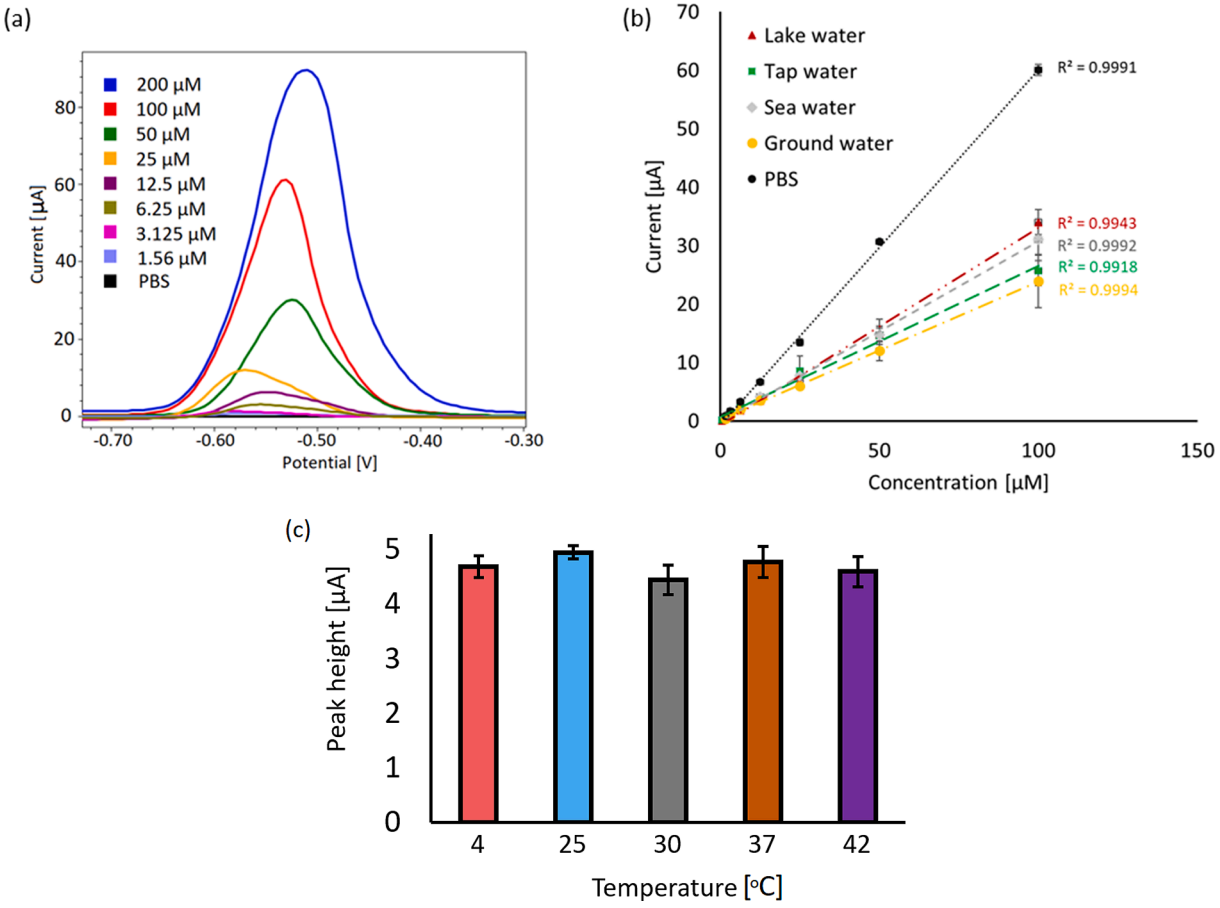

Fig. 3. Quantification of pyocyanin in water. (a) Square wave voltammograms of increasing concentrations of pyocyanin spiked in PBS. (b) Standard curves of pyocyanin in PBS, lake water, tap water, sea water and ground water. (c) Temperature effect on peak height. The extracted peak heights belonging to a constant pyocyanin concentration spiked in tap water and measured at different temperatures.

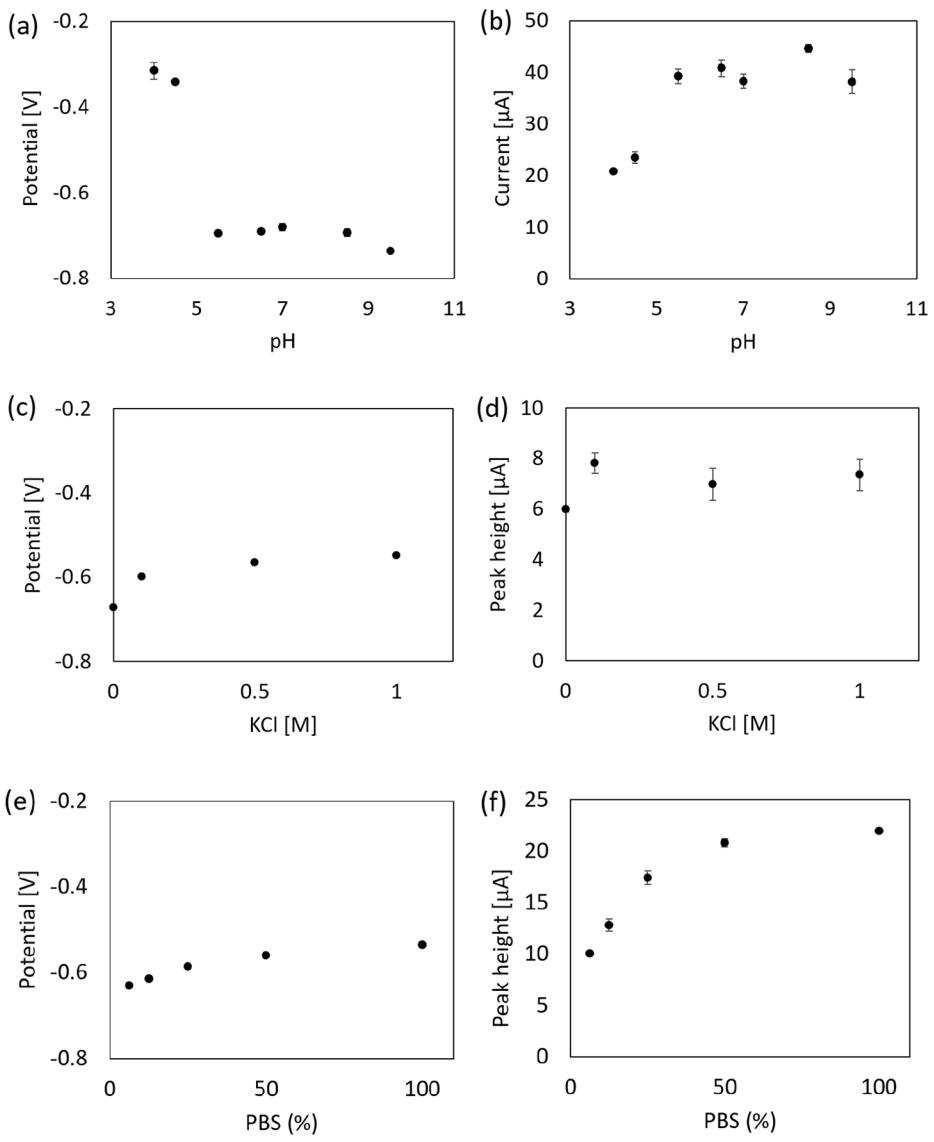

Fig. 4. The effect of pH (4.0 to 9.5) on the (a) peak potential and (b) peak current when measuring a constant concentration of pyocyanin in tap water using square wave voltammetry. The effect of the electrolyte strength on the (c) peak potential and (d) peak current when measuring a constant concentration of pyocyanin in tap water spiked with KCl. The effect of the concentration of phosphate buffer saline (PBS) on (e) peak potential and (f) peak current when measuring a constant concentration of pyocyanin in different strengths of PBS diluted with milli-Q water.

remained around −0.7 V. Likewise the peak current also showed pH dependent response (Fig. 4**b**). The peak current increased when increasing the pH from 4.0 to 5.5 and remained fluctuating around 40 μA up to a pH of 9.5. Despite the peak potential shifts and the variance in peak current as a result of the electrolyte pH, the pyocyanin signal is detectable. Most water sources have pH values between 6.5 and 8.0, which is within the range where the peak potential and peak current response is relatively stable [39].

3.4. Electrolyte strength affects peak potential and peak current

Pyocyanin was measured in tap water with increasing concentrations of KCl to investigate the effect of the electrolyte strength on the electrochemical signal (Fig. 4c and d). The peak potential shifted towards more positive potentials with increasing electrolyte concentration in the water (Fig. 4c). The peak current of the pyocyanin signal increased approximately 30 % when adding electrolyte to the water sample (Fig. 4d). However, there was no statistical difference between the peak currents of pyocyanin obtained in tap water with 0.1 – 1.0 M KCl. To test if the PBS affected the pyocyanin signal compared to the measurements in water, pyocyanin was measured directly in PBS and in PBS diluted with Milli-Q water. The peak potential shifted towards more negative potentials with more diluted PBS (Fig. 4e). The peak current of pyocyanin was also reduced with decreasing PBS content in the samples (Fig. 4f). The data support the observation that the electrolyte strength affects both the peak potential and intensity.

3.5. Detection of P. Aeruginosa in water using paper-sensors

To investigate the selectively of the method proposed in this study, the interference from *E. coli* was measured. *E. coli* was chosen, as it is a biological contaminant that also might occur in water resources. The interference was determined by adding 80 colonies/mL from an overnight culture of *E. coli* in PBS and measuring using the same SWV used to quantify pyocyanin. As seen in Fig. 5**a** no peak is detected from *E. coli*. Another 80 colonies/mL PAO1 was spiked in PBS and measured with SWV revealing an average peak height of 0.616 ± 0.025 μA (n = 3). After measuring a mix of 80 colonies/mL PAO1 and 80 colonies/mL *E. coli* the peak current remained unchanged with an average peak height of 0.574 ± 0.037 μA (n = 3). There is no statistical difference between the two values; hence no impact on the signal is observed when another microorganism is present in the sample.

The method was applied to detect *P. aeruginosa* directly in water samples by spiking 1 mL lake water with 5–50 CFU PAO1 **(**Fig. 5**b)**. There was a linear correlation between the peak height and the bacterial concentration as seen in Fig. 5**c**. The colonies had produced pyocyanin

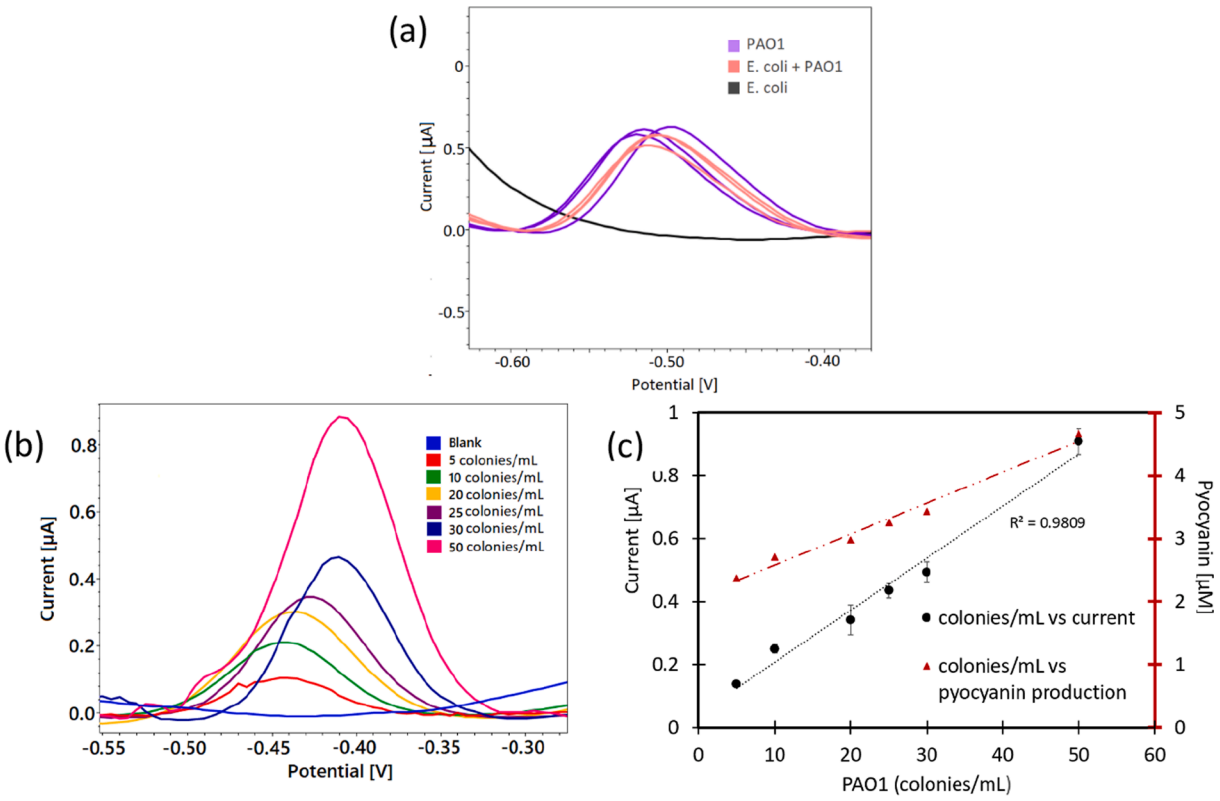

Fig. 5. Measurement of *P. aeruginosa* in water. (a) Square wave voltammetry measurements of the laboratory *P. aeruginosa* strain PAO1 measured alone and together with *E. coli* in PBS showing no difference in signal, while measurement of *E. coli* result in no peaks. (b) Measurements of PAO1 in lake water (blank) ranging from 5 to 50 colonies/mL. (c) Extracted peak heights from square wave measurements of pyocyanin plotted against the colonies/mL spiked in the water samples.

concentrations between 2.4 and 4.7 µM. The values were found by converting the obtained peak heights to pyocyanin concentrations using the standard curve of pyocyanin spiked in lake water from Fig. 2**b**. Note that the produced pyocyanin quantity is dependent on the strain genetics and the culture conditions [33].

The prevalence and concentration of *P. aeruginosa* in water varies significantly from a water source to another. K.D. Mena and C.P. Gerba (2001) has provided a comprehensive review of the *P. aeruginosa* concentrations found in different water sources [40]. In drinking water including tap water and bottled water, *P. aeruginosa* is usually at concentrations of 3–4 CFU/mL and up to 2300 CFU/mL. In whirlpools and hot tubs, the prevalence is up to 94–100 % of those tested at concentrations between $<$ 1 to 2,400 CFU/mL. Swimming pools have been reported to be contaminated with *P. aeruginosa* at concentrations ranging from $<$ 1 to 10,000 CFU/mL [41]. Recent data has shown that *P. aeruginosa* isolates from swimming pools and hot tubs are multidrug resistant in 96 % of the cases. Assuming that 1 colony consists of 5–500 cells, the concentration range detectable using the proposed method in this work is within the relevant concentration range, however, it is still not able to detect $<$ 5 colonies/mL, which is necessary for e.g., drinking water monitoring that tolerates no *P. aeruginosa* prevalence at all. Most existing methods for P. aeruginosa detection require incubation time to enhance the accuracy of the output readings (Table 2). The current electrochemical method has been applied directly after transferring the colonies to the water, thus there may be room for improvement of the lower detection limit by introducing incubation steps. However, introduction of an incubation step would exclude the use of electrochemical sensing as an on-site tool. Only PCR techniques need no incubation, however, water pretreatment steps to extract DNA/RNA and subsequent PCR processing takes several hours and are therefore not fit for on-site applications. Although electrochemical detection only takes seconds, further optimization of the lower detection range is necessary. To ensure that this technique is capable of detecting lower concentrations of *P. aeruginosa* than reported here, the measuring protocol should be optimized further. One way to accomplish this would be to up-concentrate the pyocyanin in a given sample before measurement. This could be conducted by evaporating some of the water prior to measurement.

Another important factor to study is the relation between the pyocyanin secretion, the cell concentration and the water type. In real field conditions, it may not be possible to detect *P. aeruginosa* immediately after contamination with few cells as pyocyanin is secreted upon reaching stationary phase, a time interval that can vary depending on the water composition [49]. Although, *P. aeruginosa* isolates almost always produce pyocyanin [37], different *P. aeruginosa* strains have varying pyocyanin production capabilities and therefore the measured pyocyanin concentration cannot be used as a direct measure of the cell concentration in the water. However, the more cells the more pyocyanin that can be produced and thus this method can be considered as a semi-quantitative approach for detection *P. aeruginosa*. More investigation is needed to understand in which growth phase bacteria are found in aquatic environments. Furthermore, the access of nutrients, oxygen and other chemical agents in the water is also a factor that affects the pyocyanin production and may be of interest to investigate in further studies [33,50].

4. Conclusions

P. aeruginosa is a pathogen that occasionally contaminate water sources with severe consequences for affected humans. *P. aeruginosa* is showing increasing resistance to many antibiotics which makes it difficult to treat once an infection occurs. Detection of *P. aeruginosa* in water typically involves membrane filtration, growth on a selective broth and count of visible colonies identified by the characteristic blue/green

Table 2
Examples of detection methods of *P. aeruginosa* contamination in water sources.

Detection method	Sample handling	Duration of analysis	Possibility for on-site detection	Ref
Heterotrophic plate count	Water samples streaked directly on agar	48 h	No	[42]
Real-time PCR	Water filtration and DNA extraction before pretreatment to tag primers	2–3 h	No	[43]
PCR	Water filtration and DNA extraction before pretreatment to tag primers	Not specified	No	[12]
Impedimetric technology	Incubation of samples in selective synthetic medium prior to measurements	10–24 h	No	[44]
Milliflex	Membrane filtration and image analysis together with an adenosine triphosphate bioluminescence reagent upon sample incubation	9 h	No	[45]
Fourier Transform Infrared Spectroscopy	Incubation of stationary state cells for 48 h before recording signal	48 h	No	[46]
Fluorescence based	Incubation and chemical pretreatment followed by fluorescent imaging	3–24 h	No	[47,48]
Electrochemical detection	Water samples pipetted directly on sensors	14 s	Yes	This study

pigmentation caused by pyocyanin production. Usually, this is followed up by microscopic inspection, biochemical analysis or PCR. In this work, we have reported a method for direct detection of *P. aeruginosa* in different water sources using in-house fabricated screen-printed sensors. The proposed method has the advantage of being suitable for on-site rapid detection and frequent monitoring of *P. aeruginosa* before human consumption or use of water. Further optimization of the lower detection limit is necessary before direct application in the field.

CRediT authorship contribution statement

Fatima AlZahra'a Alatraktchi: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank Kirsten Olesen and Yasemin Karatas for laboratory assistance.

Funding

No external funding has been provided for this project.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.measurement.2022.111124.

References

[1] J.P.S. Cabral, Water microbiology. Bacterial pathogens and water, Int. J. Environ. Res. Public Health. 7 (2010) 3657–3703, https://doi.org/10.3390/ijerph7103657.
[2] E.L. English, K.C. Schutz, G.G. Willsey, M.J. Wargoa, Transcriptional responses of Pseudomonas aeruginosa to potable water and freshwater, Appl. Environ. Microbiol. 84 (2018) 1–12, https://doi.org/10.1128/AEM.02350-17.
[3] WHO, Emerging Issues in Water and Infectious Disease, (2003) 24.
[4] K.G. Kerr, A.M. Snelling, Pseudomonas aeruginosa: a formidable and ever-present adversary, J. Hosp. Infect. 73 (2009) 338–344, https://doi.org/10.1016/j.jhin.2009.04.020.
[5] N. Benissa, L. Aïsse, M. Kafih, N. Zerouali, Biliary ascaris and intrahepatic lithiasis: A case report [5] [Ascaridiose biliaire et lithiase intra-hépatique: À propos d'un cas], Gastroenterologie Clinique et Biologique. 27 (2003) 1047–1048, https://doi.org/10.1038/nrmicro2204.Cholera.
[6] F.Y. Ramírez-Castillo, A. Loera-Muro, M. Jacques, P. Garneau, F.J. Avelar-González, J. Harel, A.L. Guerrero-Barrera, Waterborne pathogens: Detection methods and challenges, Pathogens. 4 (2015) 307–334, https://doi.org/10.3390/pathogens4020307.
[7] S.A. Rice, B. Van Den Akker, F. Pomati, D. Roser, A risk assessment of Pseudomonas aeruginosa in swimming pools: A review, J. Water Health 10 (2012) 181–196, https://doi.org/10.2166/wh.2012.020.
[8] https://www.who.int/publications/i/item/9789241549950.
[9] J. de Victorica, M. Galván, Pseudomonas aeruginosa as an indicator of health risk in water for human consumption., Water Science and Technology : A Journal of the International Association on Water, Pollution Research. 43 (2001) 49–52.
[10] J. Lu, I. Struewing, S. Yelton, N. Ashbolt, Molecular survey of occurrence and quantity of Legionella spp., Mycobacterium spp., Pseudomonas aeruginosa and amoeba hosts in municipal drinking water storage tank sediments, J. Appl. Microbiol. 119 (2015) 278–288, https://doi.org/10.1111/jam.12831.
[11] A.D. Brown, Some general properties of a psychrophilic pseudomonad: the effects of temperature on some of these properties and the utilization of glucose by this organism and Pscudomonas aeruginosa, J. Gen. Microbiol. 17 (1957) 640–648, https://doi.org/10.1099/00221287-17-3-640.
[12] F. Baghal Asghari, M. Nikaeen, H. Mirhendi, Rapid monitoring of Pseudomonas aeruginosa in hospital water systems: A key priority in prevention of nosocomial infection, FEMS Microbiol. Lett. 343 (2013) 77–81, https://doi.org/10.1111/1574-6968.12132.
[13] C. Hardalo, S.C. Edberg, Pseudomonas aeruginosa: Assessment of Risk from Drinking Water, Crit. Rev. Microbiol. 23 (1997) 47–75, https://doi.org/10.3109/10408419709115130.
[14] World Health Organization, Drinking Water Parameter Cooperation Project. Support to the revision of Annex I Council Directive 98/83/EC on the Quality of Water Intended for Human Consumption (Drinking Water Directive) R e c omme n d a t i o n s, (2017) 1–240.
[15] J.K. Lutz, J. Lee, Prevalence and antimicrobial-resistance of pseudomonas aeruginosa in swimming pools and hot tubs, International Journal of Environmental Research and Public, Health. 8 (2011) 554–564, https://doi.org/10.3390/ijerph8020554.
[16] E. Riera, G. Cabot, X. Mulet, M. García-Castillo, R. del Campo, C. Juan, R. Cantón, A. Oliver, Pseudomonas aeruginosa carbapenem resistance mechanisms in Spain: impact on the activity of imipenem, meropenem and doripenem, The Journal of Antimicrobial Chemotherapy. 66 (2011) 2022–2027, https://doi.org/10.1093/jac/dkr232.
[17] The Council of the European Communities, On the approximation of the laws of the Member States relating to the exploitation and marketing of natural mineral waters (80/777/EEC), Official Journal of the European Communities. (1980) 10.
[18] T.H.E. Council, O.F. The, E. Union, Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption (OJ L 330 05.12.1998 p. 32), Documents in European Community Environmental Law. (2010) 865–878. https://doi.org/10.1017/cbo9780511610851.055.
[19] H. Stender, A. Broomer, K. Oliveira, H. Perry-O'Keefe, J.J. Hyldig-Nielsen, A. Sage, B. Young, J. Coull, Rapid detection, identification, and enumeration of Pseudomonas aeruginosa in bottled water using peptide nucleic acid probes, Journal of Microbiological Methods. 42 (2000) 245–253. https://doi.org/10.1016/S0167-7012(00)00196-2.
[20] S. Caskey, J. Stirling, J.E. Moore, J.C. Rendall, Occurrence of Pseudomonas aeruginosa in waters: implications for patients with cystic fibrosis (CF), Lett. Appl. Microbiol. 66 (2018) 537–541, https://doi.org/10.1111/lam.12876.
[21] F.A. Alatraktchi, W.E. Svendsen, S. Molin, Electrochemical Detection of Pyocyanin as a Biomarker for Pseudomonas aeruginosa: A Focused Review, Sensors. 20 (2020) 5218, https://doi.org/10.3390/s20185218.
[22] M. Govindhan, B.-R. Adhikari, A. Chen, Nanomaterials-based electrochemical detection of chemical contaminants, RSC Adv. 4 (2014) 63741–63760, https://doi.org/10.1039/C4RA10399H.
[23] R. Ramachandran, T.-W. Chen, S.-M. Chen, T. Baskar, R. Kannan, P. Elumalai, P. Raja, T. Jeyapragasam, K. Dinakaran, G. peter Gnana kumar, A review of the advanced developments of electrochemical sensors for the detection of toxic and

bioactive molecules, Inorg. Chem. Front. 6 (2019) 3418–3439. https://doi.org/10.1039/C9QI00602H.
[24] B. Mojsoska, S. Larsen, D.A. Olsen, J.S. Madsen, I. Brandslund, F.A. Alatraktchi, Rapid SARS-CoV-2 Detection Using Electrochemical Immunosensor, Sensors (Basel, Switzerland). 21 (2021) 1–11, https://doi.org/10.3390/s21020390.
[25] G. Hernandez-Vargas, J.E. Sosa-Hernández, S. Saldarriaga-Hernandez, A. M. Villalba-Rodríguez, R. Parra-Saldivar, H.M.N. Iqbal, Electrochemical biosensors: A solution to pollution detection with reference to environmental contaminants, Biosensors. 8 (2018) 1–21, https://doi.org/10.3390/bios8020029.
[26] B. Pérez-Fernández, A. Costa-García, A. De La Escosura- Muñiz, Electrochemical (bio)sensors for pesticides detection using screen-printed electrodes, Biosensors. 10 (2020), https://doi.org/10.3390/bios10040032.
[27] J.S. Noori, J. Mortensen, A. Geto, Rapid and sensitive quantification of the pesticide lindane by polymer modified electrochemical sensor, Sensors (Switzerland). 21 (2021) 1–9, https://doi.org/10.3390/s21020393.
[28] J.S. Noori, M. Dimaki, J. Mortensen, W.E. Svendsen, Detection of glyphosate in drinking water: A fast and direct detection method without sample pretreatment, Sensors (Switzerland). 18 (2018), https://doi.org/10.3390/s18092961.
[29] A. Geto, J.S. Noori, J. Mortensen, W.E. Svendsen, M. Dimaki, Electrochemical determination of bentazone using simple screen-printed carbon electrodes, Environ. Int. (2019), https://doi.org/10.1016/j.envint.2019.05.009.
[30] J.S. Noori, J. Mortensen, A. Geto, Recent development on the electrochemical detection of selected pesticides: A focused review, Sensors (Switzerland). 20 (2020), https://doi.org/10.3390/s20082221.
[31] W.A. El-Shouny, A.R.H. Al-Baidani, W.T. Hamza, Antimicrobial activity of pyocyanin produced by Pseudomonas aeruginosa isolated from surgical wound-infections, International Journal of Pharmacy and Medical Sciences. 1 (2011) 1–07.
[32] J.S. et al. Alatraktchi, Fatima AlZahra'a, Noori, Paper-based sensors for rapid detection of virulence factor produced by Pseudomonas aeruginosa, P L o S One. 13 (2018). https://doi.org/10.1371/journal.pone.0194157.
[33] B. Mojsoska, M. Ghoul, G.G. Perron, H. Jenssen, F.A.Z. a. Alatraktchi, Changes in toxin production of environmental Pseudomonas aeruginosa isolates exposed to sub-inhibitory concentrations of three common antibiotics, PLoS ONE. 16 (2021) 1–12. https://doi.org/10.1371/journal.pone.0248014.
[34] F.A. Alatraktchi, H.K. Johansen, S. Molin, W.E. Svendsen, Electrochemical sensing of biomarker for diagnostics of bacteria-specific infections, Nanomedicine. 11 (2016) 2185–2195, https://doi.org/10.2217/nnm-2016-0155.
[35] D. Sharp, P. Gladstone, R.B. Smith, S. Forsythe, J. Davis, Approaching intelligent infection diagnostics: Carbon fibre sensor for electrochemical pyocyanin detection, Bioelectrochemistry. 77 (2010) 114–119, https://doi.org/10.1016/j.bioelechem.2009.07.008.
[36] J. Elliott, O. Simoska, S. Karasik, J.B. Shear, K.J. Stevenson, Transparent Carbon Ultramicroelectrode Arrays for the Electrochemical Detection of a Bacterial Warfare Toxin, Pyocyanin, Anal. Chem. 89 (2017) 6285–6289, https://doi.org/10.1021/acs.analchem.7b00876.
[37] H.J. Sismaet, A.J. Pinto, E.D. Goluch, Electrochemical sensors for identifying pyocyanin production in clinical Pseudomonas aeruginosa isolates, Biosens. Bioelectron. 97 (2017) 65–69, https://doi.org/10.1016/j.bios.2017.05.042.
[38] F.A.Z. a. Alatraktchi, M. Dimaki, N. Støvring, H.K. Johansen, S. Molin, W.E. Svendsen, Nanograss sensor for selective detection of Pseudomonas aeruginosa by pyocyanin identification in airway samples, Analytical Biochemistry. 593 (2020) 113586. https://doi.org/10.1016/j.ab.2020.113586.
[39] K. Kulthanan, P. Nuchkull, S. Varothai, The pH of water from various sources: an overview for recommendation for patients with atopic dermatitis, Asia Pacific Allergy. 3 (2013) 155, https://doi.org/10.5415/apallergy.2013.3.3.155.
[40] K.D. Mena, C.P. Gerba, Risk assessment of Pseudomonas aeruginosa in water, Rev. Environ. Contam. Toxicol. 201 (2009) 71–115, https://doi.org/10.1007/978-1-4419-0032-6_3.
[41] J. Barben, G. Hafen, J. Schmid, Pseudomonas aeruginosa in public swimming pools and bathroom water of patients with cystic fibrosis, J. Cyst. Fibros. 4 (2005) 227–231, https://doi.org/10.1016/j.jcf.2005.06.003.
[42] J. Bartram, Heterotrophic Plate Counts and Drinking-water Safety: The Significance of HPCs for Water Quality and Human Health, Water Intell. Online 12 (2013), https://doi.org/10.2166/9781780405940.
[43] C.S. Lee, K. Wetzel, T. Buckley, D. Wozniak, J. Lee, Rapid and sensitive detection of Pseudomonas aeruginosa in chlorinated water and aerosols targeting gyrB gene using real-time PCR, J. Appl. Microbiol. 111 (2011) 893–903, https://doi.org/10.1111/j.1365-2672.2011.05107.x.
[44] G. Szita, M. Gyenes, L. Soós, T. Rétfalvi, L. Békési, G. Csikó, S. Bernáth, Detection of Pseudomonas aeruginosa in water samples using a novel synthetic medium and impedimetric technology, Lett. Appl. Microbiol. 45 (2007) 42–46, https://doi.org/10.1111/j.1472-765X.2007.02145.x.
[45] Specific Detection of Pseudomonas aeruginosa in Water Using the Milliflex ® Rapid Microbiology Detection and Enumeration System, n.d.
[46] H.M. Al-Qadiri, M.A. Al-Holy, M. Lin, N.I. Alami, A.G. Cavinato, B.A. Rasco, Rapid detection and identification of Pseudomonas aeruginosa and Escherichia coli as pure and mixed cultures in bottled drinking water using fourier transform infrared spectroscopy and multivariate analysis, Journal of Agricultural and Food, Chemistry. 54 (2006) 5749–5754, https://doi.org/10.1021/jf0609734.
[47] Idexx, Pseudalert, Https://Www.Idexx.Com/En/Water/Water-Products-Services/Pseudalert/. (2022).
[48] Vermicon, VIT® Pseudomonas aeruginosa, Https://Www.Vermicon.Com/Products/Water/Test-Kit-Analysis-Pseudomonas-Aeruginosa. (2022).
[49] D. Van der Kooij, J.P. Oranje, W.A.M. Hijnen, Growth of Pseudomonas aeruginosa in tap water in relation to utilization of substrates at concentrations of a few micrograms per liter, Appl. Environ. Microbiol. 44 (1982) 1086–1095, https://doi.org/10.1128/aem.44.5.1086-1095.1982.
[50] B. Rada, T.L. Leto, Pyocyanin effects on respiratory epithelium: Relevance in Pseudomonas aeruginosa airway infections, Trends Microbiol. 21 (2013) 73–81, https://doi.org/10.1016/j.tim.2012.10.004.