Original Article

Bismuth Oxide Nanoparticles: A Bactericide That Targets The Treatment Of Contaminated Water

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Abstract

Water is considered the center of all human activity. Currently, this natural resource presents a high degree of pollution due to the growing demand and overpopulation. The existing environmental crisis has generated concern in the nations globally. The latter is causing an urgent need to search for new technologies for water management, treatment, and conservation. Nowadays, the progress in nanotechnology represents a tempting alternative for the treatment and eventual recovery of contaminated water. The application of bismuth oxide particles has become an interesting water treatment method. Their photocatalytic and photooxidative activity while interacting with organic compounds has shown bactericidal functionality, which has been acknowledged recently in the literature. This research has been built in an extensive experimental campaign based on the evaluation of the bactericidal capacity of bismuth oxide nanoparticles in water samples obtained from wastewater. The wastewater came from "San Vicente de Lacas", an Andean community in the highlands of Ecuador. The synthesis methodology applied was a green synthesis type. As a biological agent mint extract from Mentha arvensis was used. On the other hand, bismuth nitrate was used as a metal ion. The main outcome of this research was a substantial decrease in the number of colonies present in the enriched agars. The latter was evidenced as the exposure time to sunlight increased in the presence of the nanoparticles. In addition, it was observed that the pH and turbidity levels were also altered when exposed to sunlight. Therefore, this research confirms the photocatalytic bactericidal activity of bismuth oxide nanoparticles.

Keywords: Bismuth oxide nanoparticles Mentha arvensis Bactericidal Photocatalytic Bacterial resistance

1. Introduction

Water is an elementary right of every human being. According to data from the United Nations Educational, Scientific and Cultural Organization (UNESCO) around 80% of wastewater returns to the natural ecosystem without being treated or reused (Villena Chávez, 2018).

The demand for water in the world, the volume of wastewater effluents from industrial sites, and urban overgrowth are important environmental problems that generate risk in the economic and social sectors. Joint factors such as overpopulation, environmental pollution, and increased waste, combined with rigorous environmental regulations and the competition in the market are motivating researchers to look for novel technologies in the conservation and optimal management of water resources (Bagatin et al., 2014).

Ecuador is a country that has legislation applicable to water resources. Although there are several regulations in the country, there is still a problematic situation in the control of the contaminated water discharges to natural water bodies. The latter is also complemented with the lack of effluents treatment to reduce pathogens. Therefore, a vast number of lakes, canals and rivers have a high pollution level, eventually producing health problems in the surrounding populations (Caballero & Ruiz, 2015).

At the local level, in highlands countryside, the main economic activity is agriculture and most of the rural communities are supplied with water, which is used for crop irrigation. The irrigation water used for the agricultural activities of the sector has become a problem, not only for the community, but also it directly affects the entire production line that will successively be consumed by the population (Vicente et al., 2020).

Due to the high pollution in the urban population, rural areas have become clearly affected. In recent years, multiple studies have been carried out on the increase of multi-resistance bacteria, mainly at the hospital level. However, recent research has found the presence of these resistant pathogenic species in the environment. Water has become a vehicle for the dispersal of resistant species in the nature because of human activities, hospital and industrial waste, etc. Contaminated water is deposited afterwards in: lakes, canals and rivers, and hence, it is essential to look for bioremediation alternatives (Makowska, Bresa, Koczura, & Philips, 2021).

Currently, the development of innovative and efficient technologies such as alternative use for the treatment of water with high pollutant load has become a priority (Deza Martí, 2017). The use of nanoparticles in environmental treatments is taking a leading role as one of the most effective in improving the quality of effluents and extensive removal of contaminants (Chávez Lizárraga, 2018).

Metal and metal oxide nanocomposites have gained interest for their relatively small sizes and large surface areas (Ishaq, K. et al 2017). The latter can influence the direct contact with bacteria causing damage to them through the production of reactive oxygen species such as: superoxide radicals, hydrogen peroxide anions and hydrogen peroxide that interact with the cell wall (Pachaiappan et al., 2021).

Bismuth oxide nanoparticles are used in photocatalysis and for the photooxidation of organic compounds which have become two attractive alternatives for water treatment (Vargas-Hernández et al., 2016). It is considered as a stable and, at the same time, a harmless oxide; allowing its application in environmental remediation, mainly in water treatment (Mosquera et al., 2015).

Bismuth has been studied with a different perspective; recently it has been worked on a much smaller dimensional scale, the nanoscale. From there, interesting results have been obtained and some novel applications of bismuth are associated with bismuth oxides (Hernandez-Delgadillo et al., 2013), Some of the interesting applications are going to be mentioned here. Bismuth is now used as an environmental sensor in mercury reduction (Hwang et al., 2009). It has been elucidated the photocatalytic activity, in the oxidation of a dye azo Basic Red 18 (BR18) in aqueous solutions (Guler et al., 2020); in the photocatalytic degradation of tartrazine dye (TZ) exposed to ultraviolet light irradiation (El-Batal et al., 2017); and for the removal of xylene vapor from ambient air, through the process of photocatalytic degradation-adsorption by UV (Faghihi-Zarandi et al., 2020).

Various applications can be applied in synthesis. Hwang et al. (2009) did the synthesis nanoparticles using the thermal plasma and laser ablation methodology. The main drawback of the latter method is the suitability for mass production due to their low profitability and practicality. In a second phase, the authors used precipitation by applying bismuth nitrate in ammonium hydroxide, obtaining a greater amount of final product and at a lower cost.

Among the alternative and novel methods are the synthesis of bismuth oxide using honey. In this method, the honey acts as a reducing and stabilizing agent (Sharmila et al., 2019), the process is completed through the use of melanin pigment and gamma rays (25.0 kGy) at room temperature (El-Batal et al., 2017). Green synthesis by aqueous extract of Mentha pulegium has also been used (Motakef-Kazemi & Yaqoubi, 2020).

The antimicrobial activity of bismuth oxide nanoparticles has been evaluated against several microorganisms such as Salmonella, achieving a reduction of up to 70% of the bacterium growth (Firouzi Dalvand et al., 2018). Bismuth oxide nanoparticles have also an acceptable performance when the treatments included: visible light irradiation and ultraviolet light degradation. Showing in some cases efficiency up to 97.5% after 50 min of exposure to Mycobacterium tuberculosis and Salmonella (Manafi Khajeh Pasha et al., 2019).

Motakef-Kazemi et al. (2020) used bismuth oxide nanoparticles produced by green synthesis with Mentha pulegium extract. They performed their experiments to show the antibiotic action against Salmonella and Escherichia coli. Firouzi et al. (2018), determined the antimicrobial effect on methicillin-resistant Staphylococcus aureus species. Bismuth oxide nanoparticles have also been proved to exhibit antimicrobial activity against some oral microorganisms (El-Batal et al., 2017).

This research work aimed to evaluate the feasibility of using bismuth oxide nanoparticles as a bactericide in wastewater samples. The use of these nanoparticles with great potential in bioremediation has been investigated (Ferrer et al., 2018). However, minimum research has been done regarding the use of these nanoparticles as potential bactericides. At the local level, this would open several possibilities in the application of this technology to be directed towards sites with effluents that have a high microbial load. The objective of this study is to provide alternatives for the conservation of the environment, as well as to safeguard the care of water and its quality.

2. Methods and materials

Sampling Methodology

Prefiltered water samples were taken from the "Water Management Board" of San Vicente de Lacas community, which is located at Riobamba city, within the Chimborazo province. The samples were collected twice every two months. Sterilized one-liter plastic bottles were used for the collection of the water samples. Then, they were transferred to the laboratory inside a cooler tank at 4°C. The latter was done to avoid any variation due to the effects of temperature and to control cross-contamination (Casal & Mateu, 1992).

Identification of bacteria in irrigation wastewater samples

From the collected samples, diluted aliquots were taken and disseminated in nutrient culture media (blood agar and nutrient agar). Two planting techniques were used. The first technique consisted in pouring a volume of solution over the culture medium, for that case it was poured 0.1 mL of solution distributed over the culture medium with a sterilized glass hoe. The second technique was a surface planting. In order to isolate the generated colonies, once the colonies were obtained from the first seeding, a second seeding was carried out to obtain pure colonies.

Gram Staining was used for the morphological characterization of the bacteria. This technique was used to determine whether the bacteria correspond to Gram positive or Gram negative. To microbiologically characterize the bacterial species obtained, biochemical tests of the pure cultures were carried out. And finally, the resistance of the different bacterial species was evaluated using antibiotic inhibition techniques by the application of antibiograms.

Mentha arvensis extract

Mentha arvensis is a mint family specimen. In this experimental campaign, the leaves were washed with distilled water to remove dust particles. The leaves in better condition were selected after careful visual inspections one by one. Then they were cut into pieces and dried in sunlight environmental exposure.

The preparation of 20 g of extract was carried out using 200 ml of distilled water, leaves in pieces and then the mix of both components was boiled at 90 °C for 3 hours. The extract obtained was cooled to room temperature and filtered using Whatman filter paper following the methodology in (Motakef-Kazemi & Yaqoubi, 2020).

Green synthesis of bismuth oxide nanoparticles

To obtain the bismuth oxide nanoparticles, 2 g of bismuth nitrate was dissolved in 10 ml of distilled water at 90 °C. Then it was mixed with 20 ml of aqueous extract of Mentha arvensis at 90 °C applying constant stirring for 24 hours. The procedure followed the steps described in (Motakef-Kazemi & Yaqoubi, 2020).

The nanoparticles obtained were characterized by scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), and IR and UV-visible spectroscopy to determine their size, morphology and composition.

Evaluation of the bactericidal action of bismuth oxide nanoparticles

Prior to the formation of the cultures, the initial samples of wastewater were diluted with distilled water to reduce the bacterial load and avoid possible agglomeration of the colonies. To be able to make a correct count of the colonies generated. Enriched agar mediums were used: blood agar and nutrient agar.

To determine the bacterial grow inhibition, the cultures were exposed to sunlight in the presence of the nanoparticles. Bacterial growth and inhibition were evaluated in certain periods of time (0, 2, 4 and 6 hours). It was expected to obtain fewer colonies of bacteria with greater exposure to the sunlight.

In addition, the bactericidal action of the nanoparticles was determined, directly in diluted water samples. They were exposed to sunlight in the presence of bismuth oxide nanoparticles in different time periods: 1, 2, 4 and 6 hours.

The pH and turbidity of water aliquots were evaluated over time. The measurements were obtained before and after exposure to the treatments using sunlight and nanoparticles.

3. Results

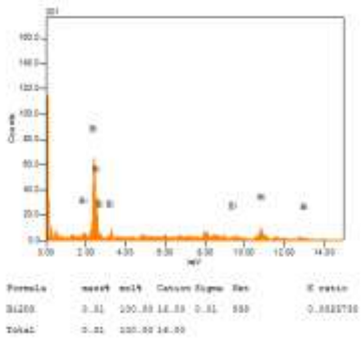
Green synthesis of bismuth oxide nanoparticles

The bismuth oxide nanoparticles obtained from the green synthesis showed a surface light-brown color as observed in Figure 1.

Fig. 1 Green synthesis final product, an extract containing bismuth oxide nanoparticles.

The nanostructural characterization was determined by scanning electron microscopy and can be observed clearly in Figure 2. The nanoparticles size ranged from 20 to 90 nanometers, and they presented a round-elongated shape (bean shape).

Fig. 2 Scanning electron micrograph of bismuth oxide nanoparticles obtained by green synthesis.

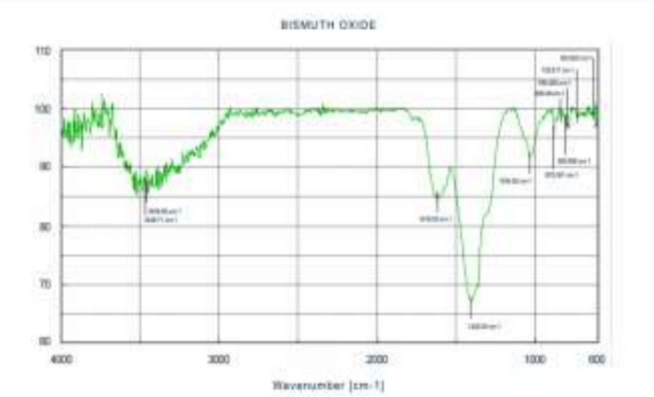


Elemental analysis by energy-dispersive X-ray spectroscopy is shown in Figure 3. The EDX analysis of the nanoparticles confirmed the presence of the bismuth peak.

Fig. 3 Bismuth oxide energy-dispersive X-ray spectroscopy.

In Figure 4 it is presented The IR spectrum of the nanoparticle sample. The spectrum shows several bands in which it is corroborated the presence of bismuth oxide. One of the vibration signals appears between 100° and 1800 cm^{-1}.

Fig. 4 IR spectroscopy of bismuth oxide nanoparticles (Bi2O3).



The UV-Vis spectrum shows a signal around 200.71 nm, compared to literature the characteristic signal of bismuth oxide is shown between 180 and 220 nm this can be seen in Figure 5.

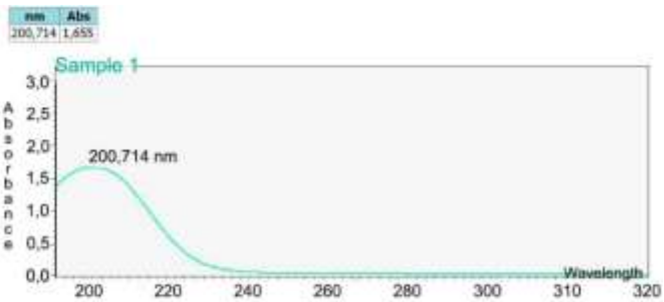


Fig. 5 UV-Visible spectroscopy of bismuth oxide nanoparticles (Bi2O3).

The material characterization in this research was done with the purpose of evidencing the presence of bismuth oxide nanoparticles. Successfully, the nanostructure of the particles it was characterized by the application of SEM microscopy. On the elemental analysis carried out by EDX confirmed the presence of bismuth in a clear spectrum. IR and UV spectroscopy techniques were also applied in order to validate the advanced characterization previously mentioned. Both spectroscopy techniques showed results that can be validated and compared in literature demonstrating the presence of bismuth oxide. Therefore, the synthesis procedure is a confident method for producing bismuth oxide nanoparticles.

The results of the characterization drove this research to test the bismuth oxide nanoparticles as bactericide agent in wastewaters. The results of the latter is described in the following sections.

Identification of bacteria in collected water samples.

The tests were performed on samples of wastewater, which was microbiologically characterized to identify bacterial species, potentially pathogenic to humans. For the typing of the species present in the specimens, samples were taken from different points, preserving the asepsis measures to avoid cross-contamination. From the samples, multiple plantings were made in enriched agars to obtain mixed cultures and subsequently isolate the individual colonies. Once all the bacterial species found were isolated, they were characterized by applying biochemical tests.

As shown in Table I, the following bacterial species were identified: Escherichia coli, Pseudomonas oryzihabitans, Klebsiella pneumoniae, Salmonella paratyphi, Shigella flexnery, Edwardsiella tarda and Staphylococcus aureus.

Table I: Results of biochemical tests and bacterial identification (+ positive and – negative)

Glucose	SH2	Lactose	Gas/Glucose	Citrate	Indole	Mannitol	Mobility	Urea	LIA	Bacterium
+	–	+	+	–	+	+	+	–		*Escherichia coli*
+	–	–	–	–	+	+	–	–		*Inactive Escherichia coli*
+	–	–	–	–	–	+	–	–	+	*Shiguella flexnery*
+	+	–	+	–	–	+	–	–		*Edwardsiella tarda*
+	–	+	+	+	–	+	–	+		*Klebsiella pneumoniae*
+	+	–	+	–	–	+	+	–	+	*Salmonella paratyphi*
+		–			–	+	+	+	–	*Pseudomona oryzihabitans*
						+				*Staphylococcus aureus*

In addition to microbiological characterization, bacterial resistance studies were carried out using antibiotic inhibition tests; multi-resistance in Edwardsiella tarda, Salmonella paratyphi, Staphylococcus aureus and Shigella flexneri. These results indicate that the dispersal of multidrug-resistant bacterial species in the environment is increasing.

Bismuth oxide nanoparticles bactericidal performance

The bismuth oxide nanoparticles were placed in mixed cultures (nutrient and blood agar mediums). The cultures were exposed to different periods of sunlight for the evaluation of the photocatalytic activity of the nanoparticles on organic matter. As can be observed from Table II, as the exposure time increased, the number of colonies in the culture media decreased. It was verified that the nanoparticles can inhibit bacterial growth after 2 hours of exposure.

In addition to testing bacterial cultures, the bactericidal activity of the nanoparticles was evaluated directly in wastewater samples. For this, pH and turbidity values were taken to corroborate the bacterial catalysis.

In Table III and IV, it can be seen the results of the tests carried out on the effect of nanoparticles on water samples in the presence and absence of sunlight. Samples that were exposed to sunlight arrived at a neutral pH in a much shorter time than samples that were not exposed to sunlight. On the other hand, the samples in the presence of sunlight significantly reduced their turbidity values as a function of time; while those that were not exposed to sunlight slightly reduced their turbidity.

TABLE II. Microbiological tests to determine bacterial growth with sunlight exposure

Sunlight exposure time	Culture medium			
Hours	Nutrient agar		Agar blood	
0		15 colonies		5 colonies
1		4 colonies		3 colonies
2		0 colonies		0 colonies
4		0 colonies		0 colonies
6		0 colonies		0 colonies

TABLE III. Parameters obtained in water aliquots exposed to sunlight.

Sunlight exposure (h)	pH (1-14)	Turbidity (NTU)
0	6.72	7.30
2	7.31	5.61
4	7.28	4.63
6	7.25	3.18

TABLE IV. Ph and turbidity parameters obtained in water aliquots without sunlight exposure.

Exposure (h)	pH (1-14)	Turbidity (NTU)
0	6.72	7.30
2	7.32	7.30
4	7.60	6.63
6	7.74	5.83

4. Discussion

The study of alternatives to reduce the impact of environmental pollution and the spread of multidrug-resistant species on the environment is currently a common aim within the scientific community. In many countries, rural population use irrigation water for their agricultural and livestock activities, water comes from rivers, springs or treated wastewater. However, these water sources present a high bacterial contamination that is contributing to the dispersion of multidrug-resistant bacteria in the population.

The use of nanoparticles as bactericidal agents is being widely studied. The production of these nanoparticles by green synthesis is encouraged to avoid environmental pollution, in addition to provide high efficiency and profitability.

In this research the green synthesis method was used using an extract of Mentha arvensis. Elemental analysis by energy dispersive X-ray spectroscopy showed the presence of bismuth within the composition of the nanoparticles. The nanoparticles have an elongated and round morphology, with a particle size ranging between 20-90 nm. The latter was determined by scanning electron microscopy. The nanostructure of the bismuth oxide nanoparticles was also studied by Firouzi et al. They determined that the morphology of the nanoparticles presented a rod/beans shape.

Microbiological tests carried out to determine the inhibition of bacterial growth in the presence of bismuth oxide nanoparticles. Experiments were done at certain periods of time with sunlight exposure. It was showed that the photocatalytic effect of nanoparticles was progressive. The photocatalytic effect increases due to the amount of nanoparticles exposed to solar radiation. As a result of it, microbial activity was affected (Table II). A similar effect is observed in water samples with added nanoparticles, when the samples were in direct contact with sunlight, the pH and turbidity parameters were normalized as the time passed (Tables III and IV).

The bactericidal mechanism of metal and metal oxide nanocomposites involves the production of reactive oxygen species including superoxide radical anions, hydrogen peroxide anions and hydrogen peroxide. These compounds interact with the cell wall of bacteria causing damage to the cell membrane while inhibiting further cell growth with leakage of internal cellular components, leading to the death of bacteria (Pachaiappan et al., 2021). This was assumed to be the bactericidal mechanism of the bismuth oxide nanoparticles.

The nanoparticles used in this research have a great advantage over other types of nanomaterials, they do not present harmful effects on the environment in the short or long term. Because of this, and to the bactericidal activity of bismuth oxide, the method developed in this work can serve to prevent the proliferation of bacterial species in commonly used water sources, even if they have antibiotic multi-resistances.

5. Conclusions

In literature, the action of bismuth oxide nanostructures has been studied, since it is a compound with a wide range of applications. The results of this research have shown that nanomaterials with photocatalytic capacity have a good performance in the degradation of certain organic contaminants.

The main characteristic found in this work about the bismuth oxide nanoparticles are the antibacterial properties against pathogenic microorganisms such as Escherichia coli, Salmonella and Mycobacterium tuberculosis; the success of the treatment is reached even though there were multi-resistance bacteria species found in this study. Through degradation experiments and microbial inactivation, an approximate of 90% of reduction was obtained. This result marked that the bismuth oxide nanoparticles are undoubtedly versatile compounds. I addition, they do not require the expense of exorbitant funding.

Finally, bismuth oxide nanoparticles are an attractive alternative for water treatment. They have shown the achievement of positive results in removing pollution and bactericidal action. In addition, it is harmless to the human system and is a method that can be used in the future for large-scale wastewater treatment, as demonstrated in this research.

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