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Comparison of ultrasound and UV technologies to control bulking and foaming in a wastewater treatment facility. A case study in an industrial park in Morelos, Mexico

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Most wastewater treatment plants use activated sludge-based biological systems for this purpose. The latter must effectively remove organic matter and, at the same time, show good sedimentability. However, sometimes there is an excessive proliferation of certain bacteria, giving rise to filamentary swelling, compromising the excellent sedimentability of the sludge. In this sense, the study's objective was to evaluate the effect of applying two different technologies, the application of low-frequency ultrasound and UV radiation. Some bench-scale experiments were performed using the bulked sludge from the secondary clarifier of a wastewater treatment facility in an industrial park (CIVAC) in Morelos, Mexico, affected by filamentous organisms. Results showed that for the UV application for two, four, and 6 min, the settleability of the mixed liquor suspended solids was not improved; on the other hand, the cavitation effect caused by the ultrasound application demonstrated effective action against the destruction of filamentous organisms. The 10 min condition showed a significant decrease in the filament integrity of the microorganisms and a significant improvement of sedimentation velocity and sludge volume index (SVI) values and settleability of the sludge, but not enough to satisfy national discharge regulations related to total suspended solids in the treated effluent. Molecular identification indicates the presence of the genera *Thauera* and *Brevundimonas* in the sludge.

KEYWORDS

filamentous bulking, foaming, advanced oxidation technologies, ultrasound, UV radiation

1 Introduction

The activated sludge system is the most common aerobic process to treat industrial wastewater. Due to its effectiveness releases a high-quality treated effluent with more than 95% of organic matter and solids removal. Under normal conditions in activated sludge, bacteria occur singly, in small chains or clumps. Under adverse conditions, certain bacterial species undergo incomplete cell division, causing the resulting cells not to separate, but remain associated with each other, generating "branches" or "filaments"; as these structures dominate the wastewater treatment system, they can interfere with settling and may cause foaming upon aeration. These conditions are expected to be present during the operation of some industrial wastewater treatment facilities due to several situations, such as the high toxicity of some specific effluents. Bulking sludge is defined as sludge with a sludge volume index (SVI) of more than 150 ml/g (Bitton, 2005). These problems could adversely affect effluent quality and is often presented as the growth of dispersed or non-settleable biomass, and a pin floc appears and alters the efficiency of the sedimentation process. As soon as these problems are detected, the goal is to control the presence of those organisms that change the settleability of the mixed liquor.

Conditions that cause bulking in a facility are described in Table 1 (Richard, 2003).

Different control techniques are employed to try to solve this problem. Two strategies exist: specific or non-specific: The non-specific methods include some oxidation techniques applying chemicals like chlorine, ozone, and hydrogen peroxide. The most common oxidant used is chlorine, which is cheap and widely available, and used by more than 50% of facilities. However, there is a fear of the organo-halogens formed and discharged in final effluents to receiving waters (Toprak Home Page, 2006; Yilmaz et al., 2008). On the other hand, specific methods are those preventive methods that aim to favor the growth of floc-forming bacterial structures against filamentous bacterial structures. The challenge is to find and maintain the appropriate environmental conditions in an activated sludge treatment plant to reach this goal. If this is impossible and the problem is present, the operator has to solve it and apply specific control methods.

Advanced Oxidation Processes (AOP), a set of non-conventional treatments, are implemented to treat domestic and industrial wastewater when conventional treatments are not effective or as a complement to these for the degradation of recalcitrant molecules such as organic micropollutants and/or inactivation of pathogens (Titchou et al., 2021). Most AOP are based on combining a strong oxidant (such as ozone or hydrogen peroxide) with a catalyst (transition metals or photocatalysts). However, some are based on electromagnetic radiation, such as ultraviolet (UV) radiation and ultrasound. In addition, the latter does not require the addition of external chemical agents to generate hydroxyl radicals (·OH), a highly oxidizing agent that can cause the oxidation of most organic compounds (Sharma et al., 2018; Titchou et al., 2021).

Ultrasound has been successfully applied to inactivate pathogens and remove contaminants present in wastewater (Vázquez-López et al., 2019), as well as for the hydrolysis of activated sludge (Al-Hoqani et al., 2022). Ultrasound transducers transform electrical current into mechanical energy and generate mechanical vibrations and acoustic cavitation (the phenomenon of growth and collapse of bubbles due to the action of acoustic waves). Acoustic cavitation simultaneously causes physical, chemical, and mechanical phenomena that cause adverse effects on bacteria and other cellular structures (David and Cheeke, 2017). On the other hand, irradiation of water with UV light in the 150–200 nm range (UV-C) can decompose water into hydroxyl radicals and hydrogen atoms (Jin et al., 2017). However, the lamps usually used in disinfection systems attached to wastewater treatment plants use a higher wavelength (close to 250 nm). These types of lamps have a germicidal action as a result of the damage caused by UV radiation to the genetic material (DNA and RNA), resulting in the death of microorganisms and its application in the disinfection of water for human consumption (Song et al., 2016) and elimination of pathogens (and organic micropollutants) in effluents from wastewater treatment plants is well documented (Masschelein and Rice, 2002; Yang et al., 2014). In this sense, both ultrasound and UV radiation could break the filamentary structures of voluminous sludge and improve the sedimentation properties of the activated sludge.

This paper aimed to study the effect of the application of UV irradiation and low-frequency ultrasound technology on the disintegration of filamentous biomass present in the mixed liquor tank of an activated sludge industrial

TABLE 1 Some conditions cause bulking in a wastewater treatment facility (Adapted from Richard (2003)).

Causative conditions
Low pH (<6.0)
Low dissolved oxygen (depending on the organic load applied)
Low F/M
Septic wastes/Sulfides (high organic acids)
Nutrient deficiency (N and, or P)
High Grease/Oil Toxicity

FIGURE 1
Suspended solids sample from the secondary clarifier.

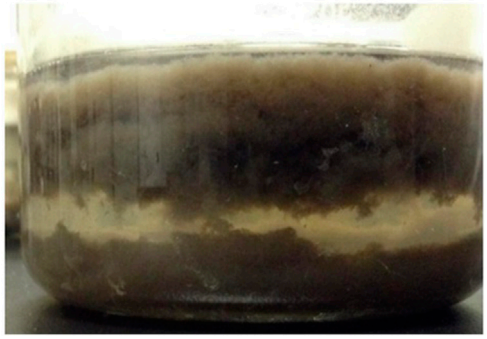

FIGURE 2
Sludge with bulking and foaming problems.

wastewater treatment plant. It is assumed that the application of these technologies will improve the settleability of the sludge, the values of the volumetric sludge index, and, therefore, the reduction of the suspended solids content in the effluent.

2 Materials and methods

2.1 Industrial wastewater (IWW) sludge sampling

ECCACIV (Empresa para el Control de la Contaminación del Agua de la Ciudad Industrial del Valle de Cuernavaca) is a private wastewater treatment facility constructed to treat the wastewater generated in an industrial corridor in the valley of Cuernavaca in Morelos, Mexico. The technology used is aerobic technology (Biotowers/Krofta) and treats 210 L/s of mixed municipal wastewater from the surrounding area and wastewater discharges coming from the industrial facilities located at the corridor.

Suspended solids samples were obtained from the secondary clarifier (Figure 1). Collection, preservation, and analysis were made weekly, following the Standard Methods procedures (APHA et al., 2005). The sampling period was during 2 months when the problem of floating and foaming in the clarifier appeared (Figure 2). The settled sludge volume was determined on-site. The Mixed liquor suspended solids determination and the filamentous bacteria observation, UV, and sonication treatment were carried out at the laboratory installations.

FIGURE 3
Diagram of the experimental set-up and the Cole Parmer. Mod. CP505 sonicator.

2.2 Experimental set-up

2.2.1 Ultrasound application

Experiments were performed using the following conditions and equipment:

a) Sonication device: A Cole-Parmer CP505 sonication equipment.
b) Operational parameters: 20kHz, 35% amplitude, and an ultrasonic density of 3333.3 W/L.
c) Sonication times: 5 and 10 min (no temperature control was used).
d) Sample volume: 160 ml of mixed liquor.

Experiments were performed in a batch system. Figure 3 shows a schematic diagram of the sonolysis reactor and an image of the equipment used. Between each application, the tip of the sonicator was washed with sterile water.

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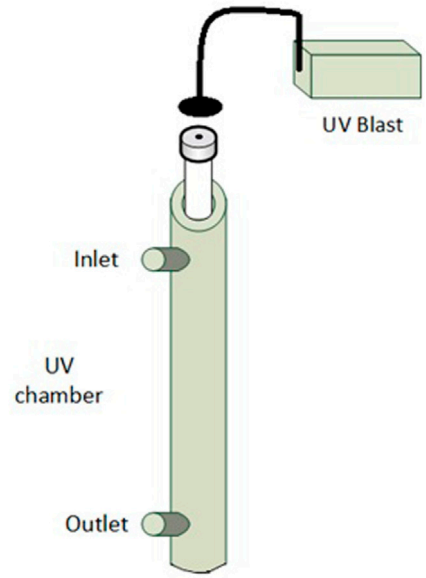

FIGURE 4
UV light experimental device used for treating mixed liquor with UV light.

2.2.2 UV light application

A commercial UV sterilization system (PROUV24-24, InstaPura, United States) was used (Figure 4). The reactor consisted of a 55 × 11 × 08 cm stainless steel cylinder containing a concentric quartz tube (TC-UV20.88). A UV light lamp (LG-UV21W, United States) with an emission length of 254 nm, an input voltage of 120 V (60 Hz), and an output power of 25 W was located inside the reactor. The treatment was applied in batch mode at a volume of mixed liquor of 140 ml for 2, 4, and 6 min. Before and after the treatment, the sonicator device was washed with sterile distilled water.

2.3 Tracking parameters

These parameters were performed before and after applying ultrasound and UV radiation to the sludge samples from the secondary clarifier.

2.3.1 Physico-chemical parameters

The content of total suspended solids (TSS) and the sludge volume index (SVI) were determined based on standard methods (APHA et al., 2005). On the other hand, total dissolved solids (TDS) and pH were measured with portable multiparameter equipment (PCSTestr 35, ThermoScientific, United States).

2.3.2 Microbiological parameters

Since two layers could be distinguished in the mixed liquor samples from the clarifier: supernatant (SN), and sediment (SD), both layers were analyzed for each sample for this test. Methylene blue was used to stain the filamentous bacteria in the sludge samples. For this purpose, 20 µl of the sample was diluted with 100 µl of distilled water, and 100 µl of the dilution was fixed on a slide and stained with two drops of methylene blue (500 mg/L). The samples were washed and allowed to dry before direct microscopic observation (ZEISS, Germany).

2.4 Identification of bacteria and molecular analysis

To obtain isolated colonies, SN and SD samples were subject to decimal dilutions using sterile nutritive broth added with wastewater (1:1) to enrich the culture medium with the components required by microorganisms; then, 100 µl of each dilution were seeded in nutritive agar plates prepared using wastewater, the liquid was spread with sterile glass beads. The seeded plates were incubated for 24 h at 30°C. To obtain pure cultures, each isolated colony was grown in nutrient broth (supplemented with sterile wastewater) for 24 h at 30°C. Subsequently, genomic DNA was extracted from each pure culture using the Genomic DNA Purification Wizard kit (Promega, United States) according to the manufacturer's instructions. Next, the amplification of the 16 S rRNA gene was carried out using the universal primers fD1 (5′-AGAGTT TGATCCTGGCTCAG-3′) and rP2 (5′-ACGGCTACCTTGTTA CGACTT-3′) (Weisburg et al., 1991). PCR Master Mix 2X (K0171, ThermoFisher Scientific) was used in a 50 µl reaction volume. First, the reaction tube was subjected to 94°C for 2 min, then to 35 amplification cycles at 94°C for 15 s, 59°C for 15 s, and 72°C for 1 min 30 s, finally to 72°C for 5 min. To assess the obtaining of the desired PCR products, electrophoresis was run on 1% agarose (90V/50 min) stained with ethidium bromide, using a DNA molecular weight marker (SM1373, ladder 2, ThermoFisher Scientific) as a reference. Finally, the DNA Clean and Concentrator-5 kit (Zymo Research, United States) was used to purify the amplicons. The purified PCR products were sent for sequencing (Sanger sequencing) to the Institute of Biotechnology of the UNAM (Cuernavaca, Mexico). The sequences obtained were analyzed with Chromas (http://technelysium.com.au/wp/chromas/) and FinchTV (https://digitalworldbiology.com/FinchTV) software, and the fasta sequences were subject to a similarity analysis using BLAST (Altschul et al., 1990) to determine the genus of the organisms. The phylogenetic analysis for the *Thauera* genus was done using the partial sequences of the 16 S ribosomal gene with accession numbers ON222725.1, ON222726.1, MW741510.1, MW647554.1, MW510006.1, NR_026153.1, AB021377.1, AB681922.1, NR_024972.1, MN538255.1, AF229887.1, AF229867.1, MH251633.1, NR_025283.1, KY287941.1, NR_027224.1, EU434525.1, EU434481.1, NR_159313.1, NR_026474.1, NR_024850.1, and the 16 S sequence obtained from the whole genome with

TABLE 2 Effect of the application of a five and 10 minutes of ultrasound and UV treatment to the bulked sludge from the secondary clarifier on SVI and TSS values in the final effluent.

	TSS effluent (mg/L)	TSS reduction (%)	SVI (ml/g)
NOM-001-SEMARNAT-2021*	60**		
PC	206.47		980
US 5	23.5	88.6	840
US 10	20.79	89.9	460
UV 2	139.40	32.4	960
UV4	81.50	60.5	960
UV6	106.9	48.2	960

PC, positive control. US, 5 and US, 10 = Ultrasound application for 5 and 10 min, respectively. UV2, UV4, and UV6, Radiation applied for 2, 4, and 6 min, respectively.
*Mexican official standard.
**Maximum limit allowed for discharge into rivers, streams, and canals (monthly average) mg/L.

accession numbers NZ_CP014646.1 and NZ_CP029331.1, the 16 S gene sequences of *Azoarcus communis* with accession number NR_024850.1 was used as outgroup.

For the phylogenetic analysis of sequences related to the genus *Brevundimonas,* the partial sequences of the 16 S ribosomal gene with accession numbers ON222727.1, KT000269.1, DQ979376.1, MK850354.1, MK034262.1, MN989047.1, MN493912.1, and the 16 S sequence obtained from the whole genome with accession numbers CP035093.1, NZ_CP021995.1, NZ_CP066026.1, NZ_CP073063.1, NZ_CP062222.1, NZ_CP015614.1, NZ_CP032707.1, NZ_CP038027.1, NZ_CP080036.1, NZ_CP080034.1, NZ_CP062006.1, NC_014375.1, NZ_LR588407.1, NZ_CP022048.2, NZ_CP067977, NZ_CP048751.1, the 16 S sequence of *Phenylbacterium parvum* (NZ_CP029479.1) was used as outgroup. The phylogenetic tree was built with the program CLC Sequence Viewer V8.0, using UPGMA as the tree construction method and Jukes-Cantor as a nucleotide distance measure, with 100 replicates as bootstrapping.

3 Results and discussion

3.1 Effect of ultrasound and UV radiation on physicochemical and microbiological parameters

SVI quantification has been used as a standard method to determine the presence of filamentous bulking conditions. This parameter is considered the volume, in milliliters, of 1 G of suspended solids after 30 min of settling. A sludge with good sedimentation has SVI values of less than 150 ml/g, while a value greater than 200 ml/g indicates that the activated sludge system has bulking problems (Eikelboom, 2000; Bitton, 2005). Based on this criterion, it can be deduced that the sample of activated sludge taken from the treatment plant (PC, positive control) has bulking problems (Table 2), which is also accompanied by a high TSS content, greater than what is recommended by local standards (SEMARNAT, 2022).

After 10 min of ultrasound application, the SVI and TSS content of the mixed liquor sludge decreased to 53% and 89.9%, respectively. This is due to the effect of acoustic cavitation caused by sound waves, which leads to the breaking of cellular structures (David and Cheeke, 2017; Al-Hoqani et al., 2022), which could be revealed by microscope observations (Figure 5), where damage to the filament structure responsible for the bulking and foaming in the mixed liquor was evident. However, when applying 5 min of sonication to the mixed liquor samples, no drastic changes in the SVI were observed (only 14%), not consistent with the TSS reduction observed (and the microscopic observations that show there is damage to the filament structures). Several studies support the use of ultrasound to carry out the hydrolysis of activated sludge (Al-Hoqani et al., 2022) and the inactivation of pathogens present in them (Vázquez-López et al., 2019).

On the other hand, when using UV radiation, although the TSS content decreased (32%–61%), the SVI values did not reduce significantly after applying said treatment (Table 2). Microscopic observations corroborate that UV radiation was inefficient in breaking down filamentous structures, and therefore there was no decrease in SVI values (Figure 5).

Although the application of ultrasound drastically decreased the TSS content in the effluent (even below the limits stipulated by the local standard) of the mixed liquor, it still cannot be considered to have good sedimentability due to its SVI values (>150 ml/g). This suggests that it is necessary to increase the power applied to the mixed liquor, increase the exposure times to the ultrasonic treatment, or apply combined treatments.

In addition to SVI and SST, changes in TDS and pH were also monitored (Table 3). TDS provides us with a measure of the degree of solubilization of activated sludge when exposed to both sonication and UV radiation. In both treatments, there was no increase in TDS; however, this is not consistent with the results of

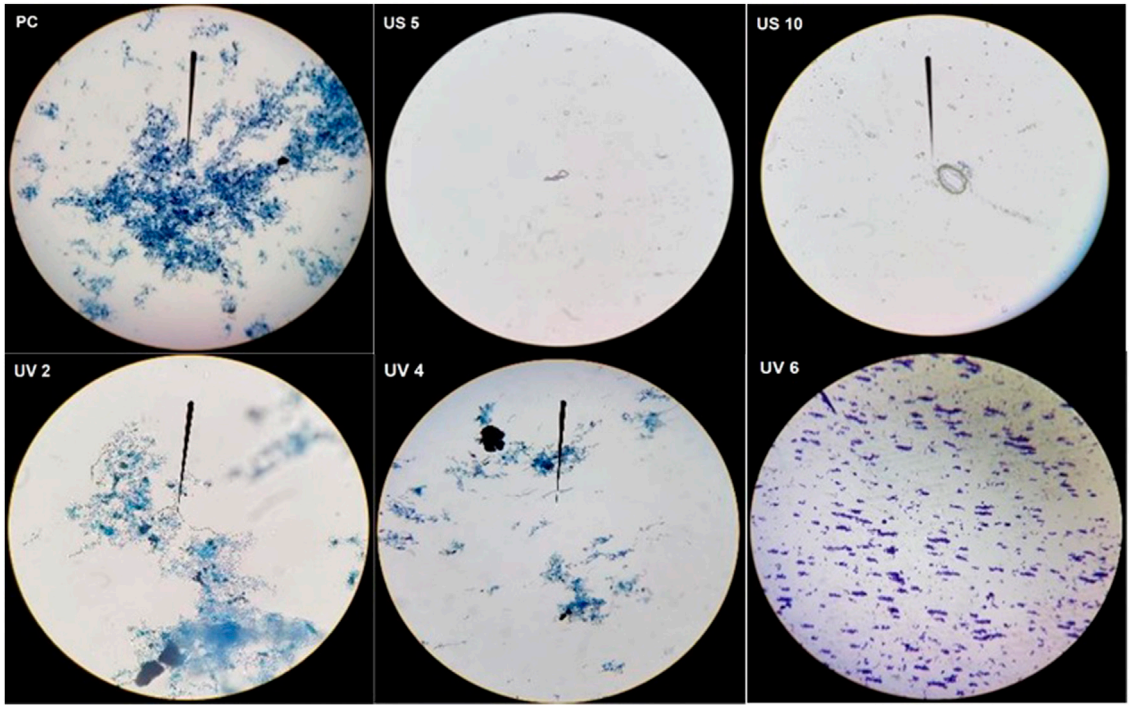

FIGURE 5
Optical microscopy of the mixed liquor before (PC) and after treatment with ultrasound (US 5 and US 10) and UV radiation (UV 2, UV 4, and UV 6).

TABLE 3 Effect of the application of ultrasound and UV treatment on TDS and pH of the mixed liquor.

Treatment	TDS (mg/L)	pH
PC	1.62	7.32
US 5	1.43	7.08
US 10	1.42	7.03
UV 2	1.41	7.54
UV4	1.38	7.20
UV6	1.40	7.20

PC , positive control. US, 5 and US, 10 = Ultrasound application for 5 and 10 min, respectively. UV2, UV4, and UV6 = Radiation applied for 2, 4, and 6 min, respectively.

TSS (Table 2), where it was observed that there is a decrease in the values of this parameter for both treatments. It is worth mentioning that these results were obtained with a multiparametric equipment, so it is necessary to carry out the tests based on the standard methods (APHA et al., 2005) to corroborate the results obtained. Instead, the typical pH range of the water that comes out of the secondary clarifier ranges between 6–8.5, according to what was obtained in the present work. Various studies have shown that pH values below 6 promote the development of filamentous bulking (Jenkins et al., 2003; Agridiotis et al., 2007) because at this pH, the growth of filamentous microorganisms is favored. On the other hand, several studies have suggested that increasing the pH from 5 to 9 decreases the SVI; this is because the bacteria leave the isoelectric state, which increases the number of active sites in the cells that leads to an improvement in floc formation potential (Agridiotis et al., 2007); however, the results are not conclusive, suggesting that the sludge settling mechanisms are more complex.

Power consumption is an essential factor to consider in large-scale ultrasound applications; However, there are few studies on this technology's use to control sludge bulking. In addition, it has also been proven that the application of ultrasound helps to simultaneously eliminate nutrients, organic matter, and pathogenic microorganisms (Vázquez-López et al., 2019); so that the use of this emergent technology can be applied for other purposes. As its application spreads, this technology could evolve into a more effective and efficient process, including lower costs.

3.2 microorganisms present in liquor samples

Sanger sequencing results of the PCR products obtained with the oligonucleotides fD1/rP2 generated three electropherograms of DNA sequences, from which DNA sequences in Fasta format

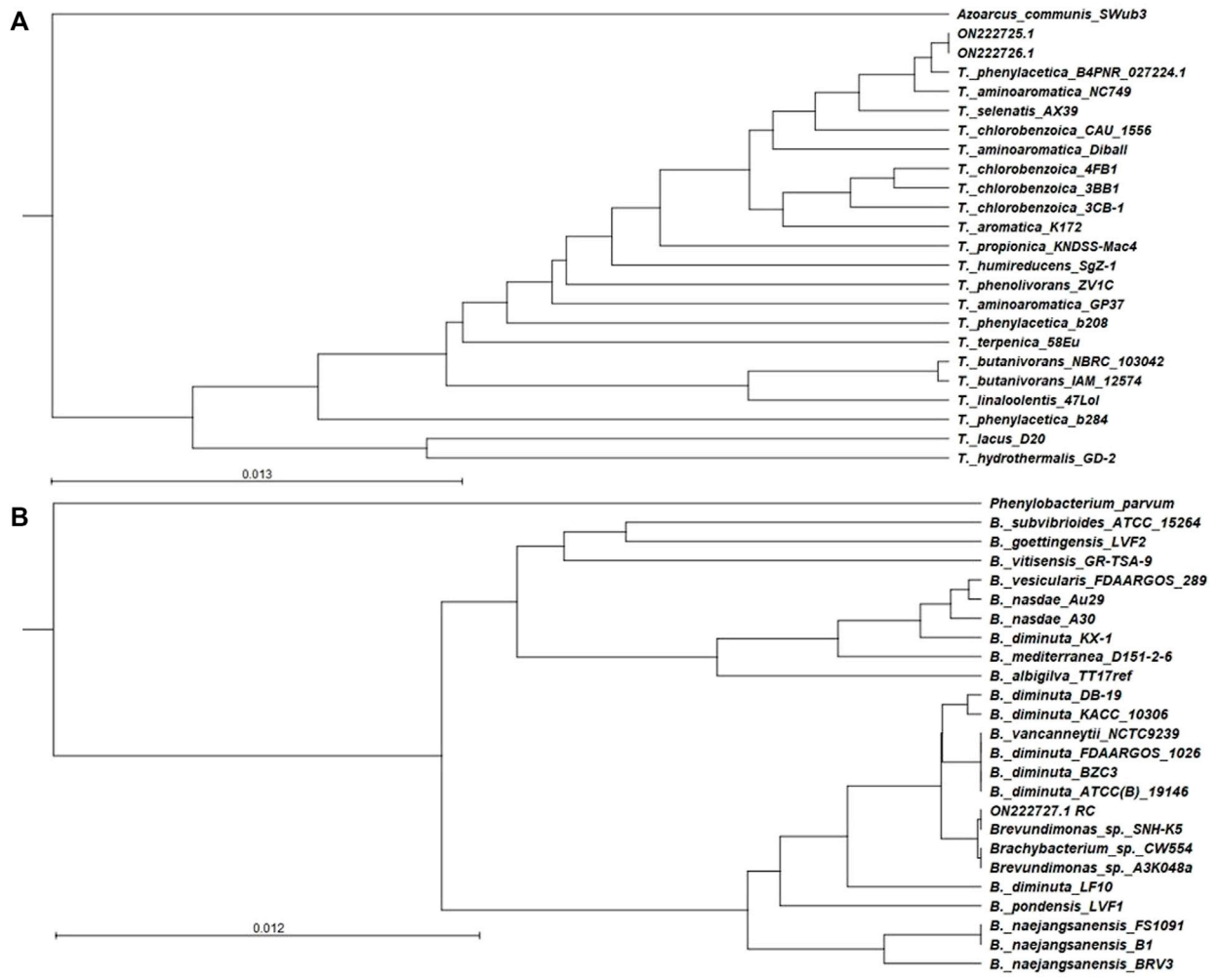

FIGURE 6
Phylogenetic trees showing the relationships of isolates ON222725.1 and ON222726.1 **(A)** and ON222727.1**(B)**.

were derived and are available through the NCBI browser under the accession numbers ON222725.1, ON222726.1, ON222727.1. A similarity analysis with BLAST showed that two of the isolations belong to the *Thauera* genus. Phylogenetics analysis was done, showing that strains NC749 and B4PNR_027224.1 of *Thaurea aminoaromatica* and *T. phenylacetica,* respectively, were the closest species (Figure 6A). The third isolation belongs to the *Brevundimonas* genus, being *Brevundimonas diminuta,* the closest defined species (Figure 6B).

Previous reports indicate that some species of *Thaurea* have been associated with incomplete denitrification and form long chains (Song et al., 2001; Liu et al., 2013; Henze et al., 2017); a possible reason that explains the formation of bulking sludge during the clarification process, as well as isolated from activated sludge (Yin et al., 2017), having metabolic capabilities allowing to degrade recalcitrant compounds as phenol. The genus *Brevundimonas* has been used to remove nitrate from groundwater (Kavitha et al., 2009); therefore, it also could contribute to the formation of bulking sludge. Strains of this genus have been previously isolated from activated sludge of coking wastewater treatment plants, with metabolic capabilities that allowed the degradation of quinoline, a recalcitrant and toxic compound (Wang et al., 2015).

Although it was possible to identify two bacterial species associated with filamentous bulking, the strategy used requires obtaining pure cultures before Sanger sequencing. This imposes some limitations: 1) loss of species that cannot be cultivated; 2) loss of bacterial species in a low proportion in the bacterial community or with high nutritional requirements; 3) sample processing time; 4) Sanger sequencing not suitable for running multiple reactions in parallel. In this sense, high-throughput DNA sequencing methodologies (next-generation sequencing,

NGS) constitute an alternative to solve the limitations as mentioned above at a lower cost (Cao et al., 2017; Slatko et al., 2018). Therefore, implementing this type of sequencing methodology is suggested to have a more complete overview of the bacterial community.

4 Conclusion

UV radiation did not damage the filamentous bacteria's cellular structure; therefore, there was no improvement in the sedimentability of the mixed liquor-suspended solids. For its part, the use of ultrasound, and the cavitation effect achieved by its application, showed a significant improvement in the sedimentability of the sludge and the SVI values as a result of the destruction of the filamentous structures; however, it is necessary to increase the application time (10 min), combine both treatments (US and UV together), or consider combining UV with hydrogen peroxide in order to comply with national regulations.

Molecular identification indicates the presence of the *Thauera* and *Brevundimonas* genera as predominant in the filamentous muds. It is suggested to use metagenomic approaches using a next-generation sequencing-based on 16 S rRNA to have a broader picture of the bacterial community, as well as whole genome sequencing of the isolated microorganisms to elucidate metabolic capabilities potentially useful for bioremediation.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

JH-R and GM-C: Conceptualization and design the experiments. MB-T, AG-G, and LT-Q: Writing and preparation of the original draft. DD: Methodology and experimentation. VB-T, JH-R, and GM-C: Resources, review, and editing of the manuscript. All authors reviewed and approved the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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