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Impact of microwaves on microorganisms colonizing diatomaceous earth after beer filtration process

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ABSTRACT

The present study aimed to assess the antimicrobial potential of microwave radiation against microorganisms isolated from diatomaceous earth, a by-product of the beer filtration process. To this end, waste diatomaceous earth from an industrial brewery was subjected to microwave radiation in a closed-chamber microwave reactor. A microbiological analysis was conducted to ascertain the presence of specific microbial groups on the diatomaceous earth prior to and following microwave treatment. The results of the tests demonstrated that microwave radiation had a pronounced antimicrobial effect on the microorganisms under investigation. Microwave treatment resulted in a time-dependent reduction of microbial abundance, with bacterial counts decreasing by 93.8–99.9% across increasing exposure times (15–60 s). The most pronounced antimicrobial effect was observed after 60 s of microwave exposure, reducing bacterial abundance to 205 CFU·g^{-1} D.M. and leading to the complete elimination of yeast. Consequently, this study broadens the application of microwaves to the eradication of microorganisms from the porous structure of diatomaceous earth, and it offers a novel approach for stabilizing this brewery waste.

Keywords: microorganisms, diatomaceous earth, brewery waste, microwave radiation.

INTRODUCTION

The majority of beers currently available on the market can be attributed to a particular style. Nevertheless, as stipulated by the Beer Judge Certification Program (BJCP), this is contingent upon the fulfillment of particular quality criteria that are specific to the style in question (Strong et al., 2021; Cappelin et al., 2024). One such characteristic is the clarity of the beer. In the case of styles such as wheat, the absence of clarity is considered a desirable trait. Conversely, in other beers such as lager, high clarity is one of the most desirable characteristics, encouraging many consumers to drink the beverage. It is therefore important to note that the presence of cloudiness in such a product will be regarded as a significant defect and may even be perceived as a potential health risk (Mastanjević et al., 2018). Moreover, it is essential to recognize that clarity can often play a pivotal role in influencing consumer purchasing decisions. This is due to the fact that it is the primary quality attribute that can be discerned even prior to the decision to consume or purchase beer (Kahle et al., 2021). Historically, cloudiness and microbial contamination were the primary quality concerns associated with beer. However, they have since become less prevalent, largely due to advancements in brewing technology (Vanderhaegen et al., 2006), including the development of effective beer filtration and stabilization processes.

It is noteworthy that the most popular method for achieving beer clarity is the use of appropriate

filtration materials. These are chemically inert substances that facilitate filtration through physical and/or mechanical means. Such materials include diatomaceous earth, perlite, cellulose-based viscose fibers, and others (Kahle et al., 2021). It is indubitable that the most prevalent filtration material utilized in brewing is diatomaceous earth, also designated as kieselguhr. The material is primarily composed of the fossilized remains of unicellular organisms, including diatoms, sponges, and other marine invertebrates (Gong et al., 2019). Due to its mesoporous structure, which comprises a multitude of diminutive pores, the utilization of this material is not confined to the brewing industry (Ide et al., 2024). Instead, it is employed in many areas of industry. In the context of brewing, however, it is the structure of the material that is employed for the removal of residual yeast cells, proteins, and sediments. It is estimated that 1 liter of clear beer generates approximately 17.14 grams of used diatomaceous earth, which equates to approximately 378.1 million kg per year generated by the entire sector (García-Díaz et al., 2024). As reported by Mei and Hui (2014), in China, 98% of breweries utilizing this material produce approximately 86,000 tons of waste annually. These considerable quantities have prompted numerous research teams to investigate effective methods for reusing used diatomaceous earth (García-Díaz et al., 2024, Semião et al., 2020).

Microwave radiation is a pervasive technology in the food industry, employed for a multitude of purposes, including food heating, thawing, drying, and pasteurization, as well as inactivation of microorganisms in food products (Woo et al. 2000). Additionally, microwaves have been employed in medical applications, including cancer therapy and as an alternative to conventional antibiotic treatments (Monserrat-Martinez et al., 2019; Zhang et al., 2022). Microwave applications undergo many regulative acts ensuring their safety and efficiency. In the case of EU, microwave technology use is regulated in dependence on the sector. In the case of processing of wastes, EU Waste Framework Directive (Directive 2008/98/EC) includes also microwave decontamination, when system should demonstrate pathogen reduction in waste handling, particularly for biohazardous materials. There are other EU regulative acts for Hygiene of Food-stuffs (Regulation EC No 852/2004) and for Novel Food Regulation (EU 2015/2283), except the effectiveness of decontamination, they include food quality and consumer safety. In United States microwave decontamination systems for waste management must meet standards of the EPA's Resource Conservation and Recovery Act (RCRA) concerning pathogen reduction and avoiding environmental hazards. In the case of food US proposed FDA standards for milk pasteurization and the Food Safety Modernization Act (FSMA) standards for pathogen reduction. There are also separate standards for Canada, Australia, New Zealand, Japan, etc. However, the global standards in the form of ISO normative are still under construction. As for the technology itself, microwaves are situated within the electromagnetic spectrum, spanning the range of 300 MHz to 300 GHz. This band can be further divided into three categories: UHF (ultra-high-frequency), which encompasses the range from 300 MHz to 3 GHz; SHF (super-high-frequency), which extends from 3 to 30 GHz; and EHF (extremely high-frequency), which includes frequencies above 30 GHz (Mumtaz et al., 2022; Kaya et al. 2020). Thermal treatment using an electric field is distinguished by its rapid generation of heat, which enables the attainment of temperatures capable of inactivating a range of microorganisms, including *Escherichia coli*, *Salmonella* spp., *Enterococcus faecalis*, *Clostridium perfringens*, *Staphylococcus aureus* and others (Kubo et al., 2020; Iglesias et al. 2024). The primary mechanism by which microorganisms are inactivated is the effect of microwaves on genes that regulate metabolism, resulting in damage to DNA and an increase in permeability and disruption of cell membrane integrity (Iglesias et al., 2024; Gedikli et al., 2008). The principal benefit of employing microwave radiation is its high efficiency, achieved through the use of minimal energy and time (Guo et al., 2017). However, this approach presents a potential drawback in the form of an uneven temperature distribution, which may give rise to the formation of cold and warm zones. This phenomenon has the potential to result in incomplete microbial inactivation, which is why it is important for microwave radiation devices to raise the temperature evenly (Popelářová et al., 2022).

The effects of microwaves on microorganisms populating diatomaceous earth following the beer filtration process have yet to be analyzed. A review of the literature reveals that only a limited number of studies have examined the effects of microwaves on microorganisms present in cultivated soil and food (Gedikli et al.,

2008; Heddleson and Doores, 1994; Valsechi et al., 2004; Brodie et al., 2015; Moline et al., 2015; Li et al., 2023). In light of the aforementioned considerations, an investigation was undertaken to ascertain whether microwave expo-sure can effectively eradicate the microbial population residing within the porous structure of diatomaceous earth, a byproduct of beer production.

The objective of this study is to demonstrate the potential for stabilizing spent diatomaceous earth for subsequent utilization, or alternatively, to halt the transformation of organic matter into fermentation and decomposition products that contribute to environmental pollution and undesirable odors, rendering the material unfeasible for storage in open air.

Our research is extremely valuable because it provides new, unique knowledge in the field of sustainable development and circular economy. The utilitarian end result of the research will be to determine how rational it is to use microwaves to reuse diatomaceous earth for filtration purposes or in other industries and agriculture. In addition, the planned research is also of a basic nature, which will allow for expanding knowledge of the properties of diatomaceous earth and the behavior of this waste in various processing processes.

Solving this problem will contribute both to increasing the potential of the waste in question, which is diatomaceous earth from the agri-food and brewing industries, but also fits into the "Green Deal" strategy in the agriculture and horticulture discipline (Transformation towards competitive equivalence "from field to table" – processing municipal waste and waste from agri-food processing using microbiological and physicochemical methods and their use for fertilizer purposes). Our research therefore responds to the need to reuse waste in accordance with the 3R (Reduce; Reuse; Recycle).

MATERIALS AND METHODS

Test material

The test material was spent diatomaceous earth. It was procured from an industrial brewery, where a 150 g·hL^{-1} blend was utilized for the filtration of beer with a 6% ethanol content by volume: Becogur 200, 1200, and 3500 (Eaton, Germany). An example of diatomaceous earth before filtration was illustrated in the Figure 1. Filtration was conducted at a pressure of 1.5 bar, with a flow rate of approximately 90 hL·h^{-1}. A sample of the earth was taken from the plate filter and averaged, then transferred to sterile 30 L containers for testing.

Test microwave

The study employed a microwave reactor with a closed chamber (Figure 2). The device had an input power of 1200 W, a microwave output power of 800 W, a microwave output frequency of 2.45 GHz, and an electromagnetic wavelength of λ = 12.24 cm. The diatomaceous earth was treated in three replicates, each lasting 15, 30, 45, and 60 s, respectively. A single sample of 25 g was placed in the chamber and subsequently transferred to a glass Petri dish with a diameter of Φ = 100 mm. Immediately after microwave treatment, the temperature of the samples was 37.5 °C for 15 s, 57.0 °C for 30 s, 68.5 °C for 45 s, and 77.8 °C for 60 s, respectively. The temperature was recorded using a DT-847U four-channel data-logging thermometer equipped with a K-type thermocouple.

The morphology of the microwave treated material was analyzed using a KEYENCE VHX-5000 optical microscope, equipped with VH-Z100R lenses. A spectroscopic analysis was conducted with a Jasco FT-IR-4200 type A spectrophotometer (Jasco, Tokyo, Japan), employing the attenuated total reflection technique with a Jasco ATR PRO ONE single reflection accessory (Jasco, Tokyo, Japan) and a ZnSe crystal. Following the microwave treatment, the diatomaceous earth was mixed and subsequently applied

Figure 1. Diatomaceous earth before beer filtration

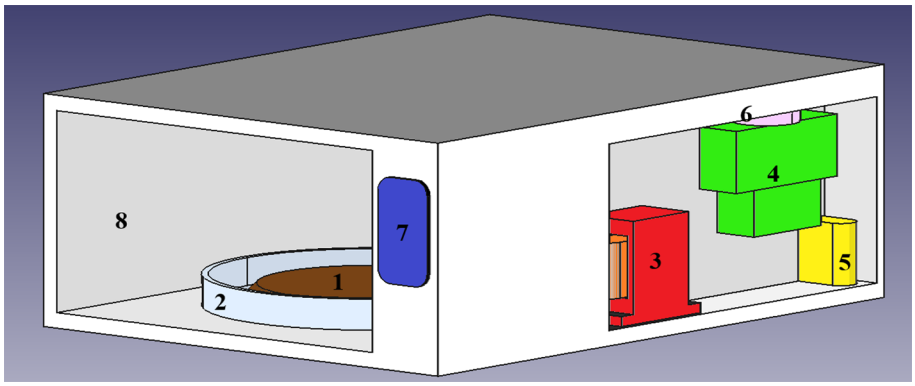

Figure 2. Simplified diagram of a closed-chamber microwave reactor: 1 – test sample, 2 – Petri dish, 3 – high–voltage transformer, 4 – magnetron, 5 – capacitor, 6 – microwave emitter, 7 – control panel, 8 – metal chamber

to the crystal, where it was compressed with a screw. The measurements were recorded in the range of 4000 cm^{-1} to 1000 cm^{-1}, with an average of 30 scans.

Microbiological analyses

All samples were subjected to microwave radiation, and control samples (not subjected to this process) were analyzed using the serial dilution method according to Koch in three replicates (Pawłat et al. 2021). The abundance of the following groups of microorganisms was determined (Table 1), with consideration of both epidemiological relevance and the use of diatomaceous earth in the beer filtration process, which would have facilitated the detection of yeast colonies. The pH of all samples was determined using a pH-meter (Elmetron, Poland) (Wolny-Koładka and Żukowski, 2019). Following the incubation period, the number of colonies that had grown was counted, and the results were reported in colony-forming units per gram of sample dry matter ($CFU \cdot g^{-1}$ D.M.).

Statistical analysis

The resulting data were subjected to statistical analysis using the StatSoft Statistica v. 13.5 software. Pearson's correlation coefficient (r) was calculated to determine the relationship between the number of microorganisms and microwave radiation time. Additionally, a one-way analysis of variance (ANOVA) was employed to assess the significance of the variation in microbial abundance across samples subjected to different microwave radiation times.

RESULTS AND DISCUSSION

The results of our microbiological analysis indicate that diatomaceous earth, a byproduct of beer production, is colonized by bacteria and yeast. In this regard, our study findings align with those of previous studies in the field, indicating that diatomaceous earth, due to its porous structure, effectively retains yeast and bacteria on its surface during the filtration process, including those that may enter the solution during routine technological work (Pilarska et al., 2022; Wang et al. 2012). The remaining microorganisms were not identified. The pH of the tested diatomaceous earth samples exhibited a neutral range (6.20–6.33), indicating an optimal environment for the growth of both microbial groups (Rousk et al. 2009). Based on these findings, it can be concluded that the pH of the diatomaceous earth has no effect on the reduction in the abundance of the microorganisms under analysis.

The results of this study demonstrate that microwaves possess strong antimicrobial properties against the bacteria and yeast tested, as illustrated in Figures 3 and 4.

In the control sample, which was not subjected to microwave radiation, the levels of bacteria and yeast were as follows: 635,760 $CFU \cdot g^{-1}$ D.M. and 1,046,722 $CFU \cdot g^{-1}$ D.M., respectively. Even with the shortest exposure time to microwave radiation, a notable reduction in bacterial and yeast abundance was observed. Furthermore, it was noted that as the duration of exposure of diatomaceous earth to microwave radiation increased, the abundance of microorganisms present decreased. The most favorable outcomes were observed following 60 s of microwave radiation, with a

Table 1. Conditions for the development of microorganisms

No.	Microorganism	Breeding ground	Development temperature	Development time
1.	Bacteria	TSA agar, BTL, Poland	37 °C	24 h
2.	Mold fungi	MEA agar, BTL, Poland	28 °C	72 h
3.	*Staphylococcus* spp.	Mannitol salt agar, BTL, Poland	37 °C	24 h
4.	*Escherichia coli*	TBX agar, BTL, Poland	44 °C	24 h
5.	*Salmonella* spp.	SS agar, BTL, Poland	37 °C	24 h
6.	*Clostridium perfringens*	SC agar, BTL, Poland	37 °C	24 h
7.	Yeast	YPD agar, BTL, Poland	28 °C	48 h

gradual decline in the abundance of bacteria and yeast to 205 CFU·g^{-1} D.M. and 0 CFU·g^{-1} D.M, respectively. The results of the analysis indicate that the percentage decrease in bacterial abundance relative to the control was 93.8% (15 s), 96.7% (30 s), 99.8% (45 s), and 99.9% (60 s), respectively. Conversely, the per-centage decrease in yeast abundance was 99.4% (15 s), 99.4% (30 s), 99.9% (45 s), and 100% (60 s).

A comparison of the processing efficiency for different treatment times is presented in Table 2. For the shortest time, the total energy supplied to the system was 12 kJ, which, when converted to the sample mass (25 g), corresponds to an energy density of 480 kJ kg^{-1}. For this time, the best coefficient of energy required to reduce the number of organisms by one logarithm was obtained, as well as the lowest times required for this reduction (D-value). For longer times, increasing the time resulted in an almost linear maintenance of these coefficients, which were on average 530/380 kJ·kg^{-1}·log^{-1} and 16.5/11.9 s·log^{-1} for bacteria and contaminants, respectively. Compared to other methods, such as the use of high temperature or UV radiation, the treatment carried out is characterized by a rapid reduction of harmful microorganisms, but with a relatively high energy demand.

The present study is in accordance with the findings of other authors regarding the potent antimicrobial properties of microwaves, which have been demonstrated to result in a neartotal reduction of microorganisms in the analyzed material. In a study conducted by Li et al. (2023), a reduction in the abundance of microorganisms inhabiting clay soil was observed following the application of microwave radiation (frequency: 2.45 GHz, power: 2 kW) for a duration of 12 minutes. The observed reduction ranged from 38% to 52%. Furthermore, the shorter microwave exposure times (3 minutes and 6 minutes) did not alter the species diversity of the identified bacteria in comparison to the control samples that were not subjected to radiation. Boumarah et al. (2016) demonstrated that the complete elimination of microorganisms from the sample could occur more rapidly, after 7 minutes. This is a considerably inferior result to that obtained in the present study. It is regrettable that the authors did not provide the power of the microwave, which has a substantial impact on the process of microbial inactivation (as the power increases, microorganisms are inactivated more rapidly). Brodie et al. (2015) demonstrated that the thickness of the soil (test material) is a crucial factor influencing microwave penetration and its antimicrobial effect. The authors subjected agricultural soil to microwave radiation (with a frequency of 2.54 GHz and a power of 2 kW) for 30 s, 60 s, and 120 s. The analysis revealed that the application of microwave treatment significantly reduced the population of microorganisms present in the upper layers of the soil (up to 5 cm), while no

Table 2. Processing efficiency for different treatment times

Treatment time, s	Energy density, kJ/kg	Energy required for 1-log reduction, kJ/kg/log		D-value, s/log	
		Bacteria	Yeast	Bacteria	Yeast
15	480	357.677	187.183	11.177	5.849
30	960	535.112	351.394	16.722	10.981
45	1440	520.272	409.730	16.259	12.804
60	1920	532.862	-	16.652	-

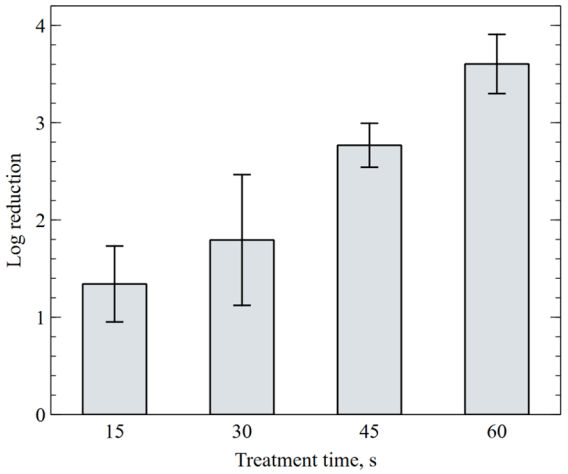

Figure 3. Logarithmic reduction in the number of colony forming units of bacteria for different treatment times (presented as mean values with standard deviations)

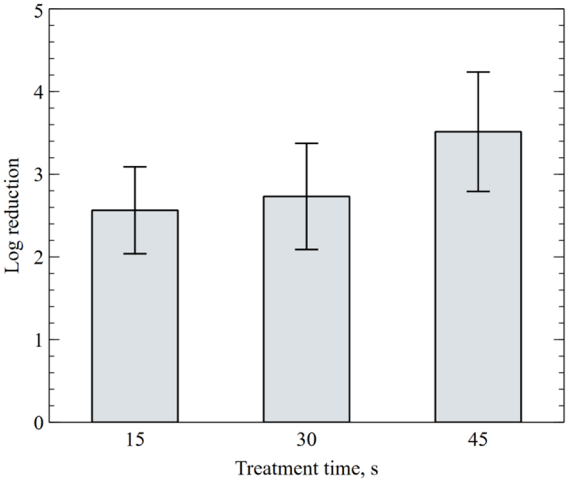

Figure 4. Logarithmic reduction in the number of colony forming units of yeast for different treatment times (presented as mean values with standard deviations)

discernible change was observed in the deeper regions of the test material. As demonstrated by Moline et al. (2015), microwave radiation can effectively eradicate 100% of microorganisms in a shorter period of time, a result that is analogous to the findings of our study. Moline et al. (2015) investigated the impact of microwave radiation on the population of microorganisms residing within honey. Mesophilic bacteria, fungi, and yeast were subjected to 800 W of radiation for 45 and 90 s, respectively. The number of mesophilic bacteria exhibited a notable decline following the process, with a 51% reduction observed at 45 s and an 80.2% reduction at 90 s. In contrast, the abundance of fungi and yeasts was more susceptible to microwave radiation, with a 98% reduction observed at 45 s and no further detection after 90 s. Gedikli et al. (2008) conducted a study to investigate the impact of microwaves on the viability of select Gram-negative and Gram-positive bacteria, including *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus*. The authors observed that at an output power of 360 W and a frequency of 2.45 GHz, the cells of the bacteria under study were inactivated within the first 60 s, reaching a temperature of approximately 70 °C. Following this initial period, no significant changes in the number of viable cells were noted. Ahmed et al. (2022) subjected *Escherichia coli* and *Staphylococcus aureus* bacteria, isolated from sheep's milk, to microwave radiation (at 1400 W). The microorganisms were subjected to radiation at 560 W, 1120 W, and 1400 W for varying time periods: 0 s, 5 s, 30 s, 60 s, and 120 s. The results of the tests indicated that the lethal effect of the radiation was contingent upon an increase in both the power level and the exposure time. At 560 W, the reduction in *E. coli* counts was 12.1%, 46.9%, 94.7%, and 96.6% after 5, 30, 60, and 120 s, respectively. In comparison, *S. aureus* counts decreased by 55.9%, 64.0%, 73.6%, and 82.5% in relation to the control group. The percentage of microwave radiation reduction at a power level of 1120 W was 48.4%, 90.1%, 97.8%, and 100% for *E. coli* after 5, 30, 60, and 120 s, respectively, compared with the control group, while the corresponding figures for *S. aureus* were 92.7%, 97.2%, 98.2%, and 99.08%. The percentage of microwave radiation reduction at a power level of 1400 W was 95.1%, 96.4%, 98.7%, and 100% for *E. coli* after 5, 30, 60, and 120 s, respectively, compared with the control group, while the corresponding figures for *S. aureus* were 80.7%, 95.7%, 98.6%, and 99.1%. It can thus be concluded that an increase in microwave radiation power results in enhanced microbial killing efficacy. Furthermore, the findings of Jankovic et al. (2014) support the hypothesis that as the power and duration of radiation exposure increase, the abundance of microorganisms declines, potentially leading to their complete elimination.

Structure of sedimentary material such as diatomaceous earth consists of silica confined diatom fragments: multi-shape (cylinder, disc, star, etc.), micro-porous (up to 1 micron) frustules. Amorphous silica ensures durability in terms of

rigidity and strength, pores provide good absorption features and light weight of material. Microscopic observations conducted with an optical microscope indicate that microwave treatment does not significantly alter the morphology of diatomaceous earth. This sedimentary rock, rich in silica, has undergone extensive geological transformations, often involving high pressures and temperatures. Therefore, the proposed treatment did not induce severe structural changes. Intensive drying occurred with the extension of the treatment time. Figure 5 compares images of diatomaceous earth rich in liquid fraction, with varying grain sizes from both the control sample and the 60-s microwave-treated sample.

The FTIR spectrum of diatomaceous earth is marked by the defining peaks of amorphous silica, particularly those related to Si-O-Si stretching and bending. Signature of silica is the asymmetric stretching of Si-O-Si bonds around 1100–1200 cm^{-1} with sometimes overlapping bands for silica structural variants. At much lower frequency, sharper peak is an indicator of Si-O bonds symmetric stretching and bending vibrations of Si-O-Si bonds. Peaks associated with water content (from adsorbed or structural water) and minor organic materials can also be seen, depending on the sample's origin and processing. There is a broad band near 3200–3500 cm^{-1} associated with O-H stretching vibrations from hydroxyl groups and adsorbed water. Bending vibration of water molecules appear near 1630–1650 cm^{-1}. Weak peaks from organic residues such as C-H stretching also can be observed (2850–2950 cm^{-1}). Potential silanol groups peaks (Si-OH) have lower wavenumber than observed. The spectrum displayed peaks characteristic of diatomaceous earth, as outlined in Table 3. The FTIR spectrum for plasma treatment does not indicate the appearance of new functional groups, but only intensity changes in some ranges. The intensity of the band in the 3100–3600 cm^{-1} range, attributed to stretching vibrations of –OH groups (silanols) and physically adsorbed water, decreased, and the absorbance at 1640 cm^{-1}, corresponding to bending vibrations of water, also decreased. These phenomena indicate effective pore dehydration and partial dehydroxylation of the sample surface. Simultaneously, the intensity of the band around 1070 cm^{-1}, characteristic of asymmetric stretching vibrations of Si–O–Si bridges, increased, reflecting the condensation of silanol groups into a more developed siloxane network. Decrease in sample volume and moist content was observed, shifting the optimum condition for microorganisms cultivation into more harsh environment (Figure 6).

The findings of this study were corroborated by statistical analysis. The differences in microbial counts between the proposed microwave exposure times were found to be statistically significant. Furthermore, statistical analysis demonstrated a strong negative correlation between the

Table 3. FTIR peaks characteristic for diatomaceous earth

Wavenumber, c^{m-1}	Description
3100	Absorbed water H-O-H stretching vibrations
3600	Absorbed water H-O-H stretching vibrations
1640	Absorbed water H-O-H bending vibrations
1070	Si-O-Si bonds: Si-O stretching vibrations

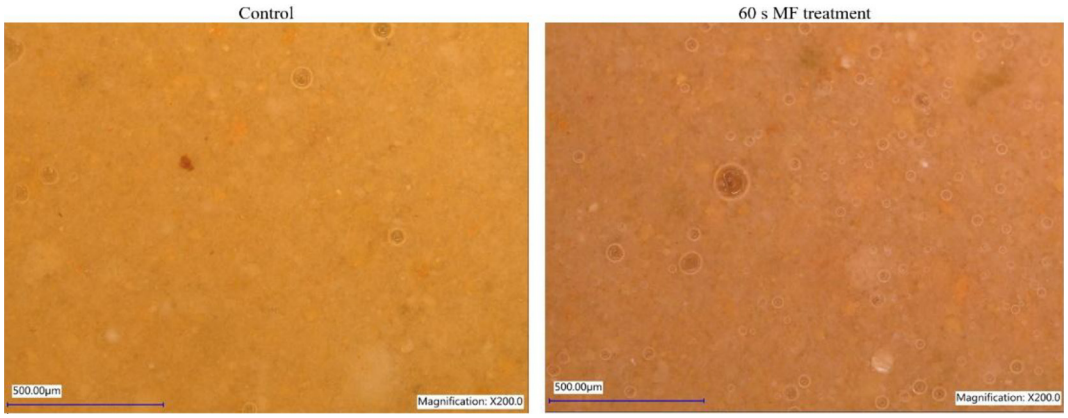

Figure 5. Microscopic imaging of diatomaceous earth before and after microwave treatment – KEYENCE VHX-5000 optical microscope, magnification 200×

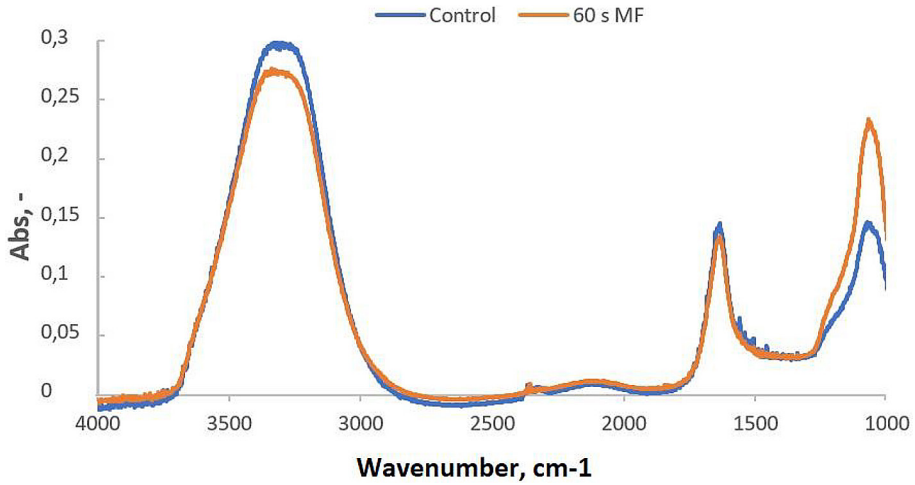

Figure 6. FTIR spectra for the control sample and the 60 s microwave treatment

average microbial abundance in the samples and the microwave exposure time. The correlation coefficient was r = -0.86 for bacteria and r = -0.97 for yeast ($p<0.05$).

CONCLUSIONS

In conclusion, the results of the conducted study confirm the effectiveness of micro-wave application in removing microorganisms colonizing diatomaceous earth after the beer filtration process. A statistically significant reduction in the number of bacteria and yeast is evident for the shortest time (15 s) and increases with treatment time, reaching a maximum reduction of 3.6 log CFU·g^{-1} D.M. for bacteria and complete elimination of yeast for 60 s of treatment. To the best of our knowledge, there are currently no published study results on the feasibility of using microwave radiation to remove microbial contaminants from the porous surface of diatomaceous earth and biologically stabilize it. Therefore, our analysis fills this gap and allows us to expand the possibilities of microwave application to new areas. However, these results reflect controlled laboratory conditions, and future studies should evaluate the process under real-world or industrial conditions to fully assess its practical potential. These findings suggest that microwave irradiation has strong potential as a physical stabilization method for brewery waste and for microbial decontamination of porous materials. It also leads to evaporation and reduction of the moist content in the treated sample, thus, may help in the reduction of waste volume. However, technology faces some limitations, especially in upscaling the process, which will require closed shielding chamber and development of adequate microwave power source. Future research should include molecular-level analyses of the microbiome, identification of non-culturable microorganisms, evaluation of alternative physical treatments such as low-temperature plasma, and assessment of scalability, energy efficiency, and industrial applicability of microwave-based stabilization processes

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