Journal of Engineering Technology and Applied Sciences, 2022 *e-ISSN:* 2548-0391
Received 9 December 2021, Accepted 23 January 2022 *Vol. 7, No. 1, 31-44*
Published online 21 March 2022, Research Article *doi: 10.30931/jetas.1034671*

Citation: Kuşku, H., "Effects of Exposure Time of Sonication on Physical Dispersal of Mucilage: A Preliminary Study". Journal of Engineering Technology and Applied Sciences 7 (1) 2022 : 31-44.

EFFECTS OF EXPOSURE TIME OF SONICATION ON PHYSICAL DISPERSAL OF MUCILAGE: A PRELIMINARY STUDY

Halit Kuşku

Canakkale Onsekiz Mart University, Canakkale, Turkey
halit@comu.edu.tr

Abstract

In recent years, severe mucilage formation threatening nearshore marine ecosystems has intensified investigations on possible separation of components forming mucilage flocculation, deactivating bacteria adhesion and decomposing the colloidal structure. Challenges to eliminating mucilage formation in marine ecosystems require long-term measures, however quick reaction with environment-friendly approach is of great importance for the control of mucilage expansion since the impact of mucilage can be significantly hazardous in nearshore marine areas during seasonal change and may spread to more expansive areas when disregarded. In the present study, ultrasonic vibration at 40 kHz frequency generated by sonication showed a time-dependent destructive effect on the colloidal structure of mucilage. Results showed that an ultrasound wave with 40 kHz frequency for 60 minutes of application could be effective for nearly 50% dispersal of mucilage aggregation on sea surface that in terms might be a useful tool for rapid response in an Emergency Action Plans. However, further research is encouraged for understanding how sonication mitigates the aggregation of phytoplankton and bacteria forming the complex matrix of polymeric mucilage structure.

Keywords: Acoustic energy, biofouling, mucilage, sonication, sound exposure time, ultrasound

1. Introduction

The formation of mucilage in nearshore marine ecosystems has been reported for many years ago in different areas around the World [1-3]. Some recent reports have documented mucilage events in the Mediterranean Sea since the 1980s [4, 5]. Several earlier studies reported diatom species (*Skeletonema costatum* and *Cylindrotheca Closterium*) as the main reason for mucilage formation [1, 6-8], while others pointed on dinoflagellates [9]. Other researchers reported high organic carbon levels in cellular secretions, mainly in carbohydrates, as a reason for mucilage occurrence [2, 3, 5]. It was underlined that mucilage can be the evolutionary product of smaller organic matter aggregates because the two subfractions always showed high compositional and structural similarities [10]. Stratification of the water column in high temperature conditions may provide a favourable ambient for the increasing coalescence of small-sized aggregates, the so-called "marine snow", that is ever-present in the oceans all around the World [11]. These compounds are high-level organic matter aggregates in the marine ecosystem, basically formed by extracellular polymeric substances released through decaying organisms or other organic matter coupled with dead plankton, diatoms, fecal matter, bacteria, and high levels of nutrients penetrating the oceans through several paths [12-14]. With time, these aggregates can form huge blankets, thin layers, and flocs, forming the mucilage [15] altogether through enzymatic activities of prokaryotic bacteria [16, 17]. Suppose the equilibrium between degradation and aggregation reactions of organic matter caused by the persistence of anoxic conditions becomes unbalanced. In that case, the complex formation of mucilage can occur [10], as a booming progressive stage of marine snow with a size ranging from a few millimeters to several meters [11], that in terms may threaten huge areas along the coastline [14, 18-20], with significant economic impacts and social concern.

Sound treatment in water ambience uses waterborne acoustic energy and sounds with frequencies below 1 kHz are classified as low-frequency sound [21]. Sound is often used for the acoustic energy range of 20Hz - 20kHz, which is audible for the human ear [22]. Ultrasounds are sound waves above 20 kHz (20kHz - 1GHz), which are not audible for humans [23, 24]. Vibration treatment uses solid-borne acoustic energy in mechanical structures such as pipes, walls of containments, etc. [21]. Successful findings regarding detrimental and destructive impacts of a wide range of acoustic energy (sound or vibration) on several forms of biofouling organisms have been reported earlier [25-29]. Microbial inactivation by ultrasonic treatment (> 20 kHz) was reported [30], with detrimental effects on bacterial growth [31, 32]. Ultrasound may potential promote or damage effects on enzymes, substrates, the reactions between enzymes and surrounding substrates [30, 33]. Earlier reports tested a wide range of audible sound frequencies from 30-100 Hz [26, 27, 29, 34, 35] to 445-5.000 Hz [26, 29, 35], and others used ultrasonic frequency range from 17-30 kHz [26, 36-38] to 63-102 kHz [38] for the control of biofouling organisms such as tubeworms, bryozoans, ascidians, barnacles, oysters, and algae. In a detailed investigation [21], it was noted that ultrasonic cavitation with frequencies between 20 and 42 kHz can be used as a destructive measure in biofouling control of mussel infestation, based on earlier reports [39, 40].

Considering various sound energy impacts on biofouling organisms, acoustic energy could be a valuable method for biofouling control. Nevertheless, information about the effects of acoustics on biofouling development remains inconsistent, and more research is necessary to understand and develop adequate strategies of biofouling control using acoustics [41]. Additionally, sound energy could be a practical tool and easily applicable method in the challenge with mucilage formation, as mobile sound generator installations can be easily carried and applied in different locations [42].

However, to our knowledge, so far, there is no published information available regarding possible impacts of acoustic energy on the control mechanism, dispersal or mitigation of mucilage formation in the marine ecosystem. Therefore, based on earlier success in microbial inactivation by ultrasonic treatment with above 20 kHz frequency [30], and the destructive effect on the biofouling of mussel infestation using ultrasonic cavitation between 20 and 42 kHz frequency [21]. The present study aimed to investigate increasing levels of exposure time of 40 kHz frequency sonication on possible dispersal of mucilage formation, as a practical and environment-friendly control measure.

2. Material and Method

This study has been done by the permission of Turkish Ministry of Agriculture and Forestry (Ankara - Turkey) (E-67852565-140.03.03-3911695). Mucilage samples were randomly collected from the Strait of Canakkale (Canakkale Marina, Turkey; 40°09' 00" N-26°29'02" E) during a mucilage formation event in the Sea of Marmara and the Turkish Straits (Istanbul-Bosphorus and Canakkale Strait) in 2021. Triplicate samples of mucilage layer were directly taken into 10 L volume plastic drums with sealable cover (Fig. 1), and immediately transferred to the laboratories of Marine Technology Engineering Department, Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University (Turkey), and stored in ambient condition without sunlight penetration. Samples taken from the Canakkale Strait were delivered to the laboratory within 25 minutes. Water quality parameters of temperature, salinity and pH was recorded applying an automatic water quality measuring device (brand of YSI), and resulted as respectively: 16,4 °C, 27,9 ppt , and 8,03.

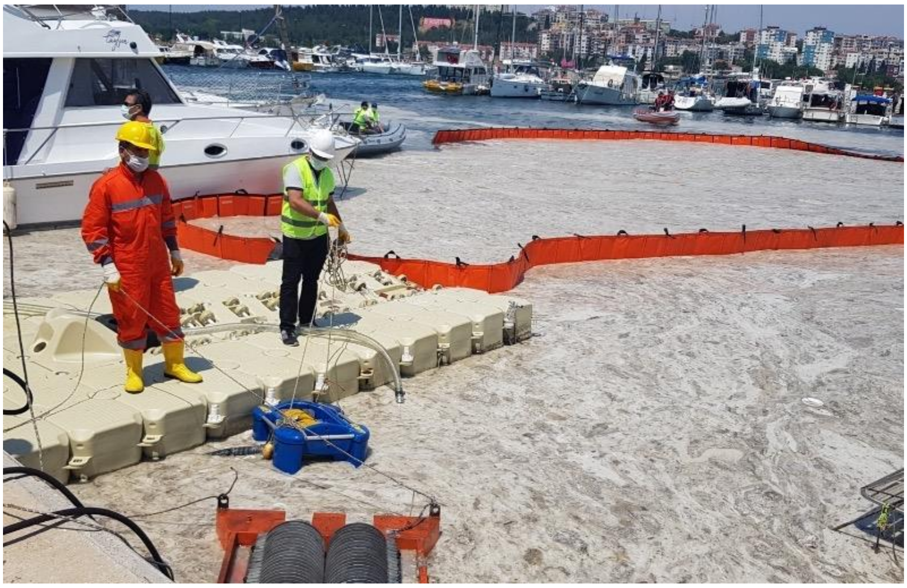

Figure 1. Mucilage event in Canakkale Strait (Turkey) and sampling area

Before ultrasonic treatment, random samples of mucilage collected from the main stock and transferred into 100 ml beakers were used for image capture of mucilage structure under light microscope (OLYMPUS CX21, 10X/18, magnification 4 x 0 10) and captured images of initial samples without treatment were recorded. After that, mucilage samples in 100 ml beakers were exposed to ultrasonic waves using a "Wisd" brand WUC-A 03H Model Ultrasonic Vibrator. The study was carried out in triplicates at room temperature (22°C) and ambient daylight. The temperature of distilled water in the sonication device was measured thoroughly and replaced with new distilled water to keep the water temperature at a constant level, the same as the room ambience of 22 ± 1°C. The temperature has been reported to influence the effectiveness of ultrasound waves on yeast cells [43, 44], and also mucilage flocculation is strongly dependent on water temperature [45, 46].

The mucilage structure was exposed to sonication at 40 kHz frequency for three different time intervals of 15, 30 and 60 minutes. A group with no acoustic treatment served as a control. After each time interval, samples were image captured under the light microscope at the same magnification mentioned above, and mucilage samples were volumetrically evaluated to compare per cent ratio between water to mucilage media on the surface. At each time interval, any possible signs of dispersal of the aggregates were examined until an apparent dispersal of aggregates through image tracking was observed.

By the end of the 60 minutes of sound exposure, all samples were kept at room temperature (22℃) for one day (24 h) and after that for another three days (96 h), namely 4 days in total for further evaluation of possible alteration of the proportional structure of water to mucilage formation (W:MF) to figure out any possible re-flocculation of aggregates after a certain period of storage.

3. Results

Mucilage structure exposed to sonication at 40 kHz frequency altered in terms of the gradual decrease in the thickness formed on water surface in a time-dependent manner with the increase of exposure time over the 60 min treatment period. The volumetric proportion of W:MF altered with increasing water and decreasing mucilage layer from the initial W:MF ratio of 10:90 to 40:60, 50:50, and 60:40 after sonication for 15, 30, and 60 min, respectively. The control group without acoustic treatment remained similar (10:90) without alteration after 60 min of exposure (W:MF, 10:90) (Fig. 2). Exposure to acoustic energy showed a 30% reduction in the surface structure with a treatment duration of <15 min and displayed a 50% reduction in the surface layer after 60 min ultrasound treatment with 40 kHz frequency (Fig. 2).

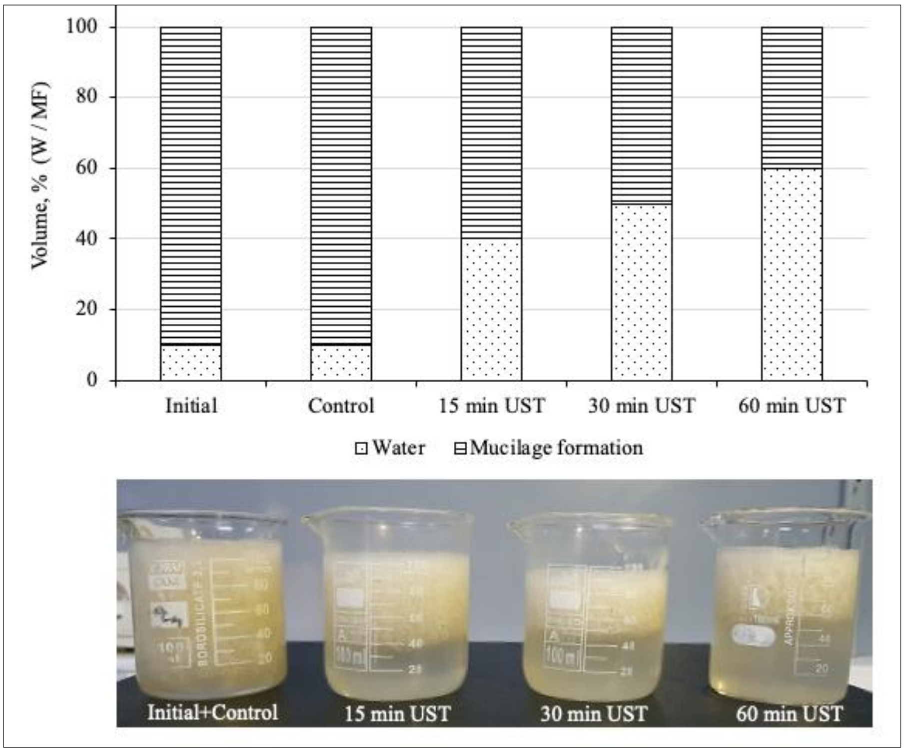

Figure 2. Time-dependent volumetric alteration of water to mucilage ratio after exposure to sonication at 40 kHz frequency. Control group without acoustic treatment.

After consecutive room storage for 24 and 96 h at 22 ℃, mucilage samples did not show any alteration in terms of volumetric proportion of W:MF, which remained similar to those exposed to sonication, with proportional contribution of 40:60, 50:50, and 60:40 for the 15, 30, -and 60 min exposure treatment groups, respectively. The only difference was seen in the control without sound treatment that increased its water level by 10% over the initial, and presented a W:MF ratio of 30:70 by the end of both 24 (Fig. 3) or 96 h (Fig. 4) storage. Microscopic image captures of the mucilage structure exposed to sonication at 40 kHz frequency level for 15, 30, and 60 min are given in Fig. 5.

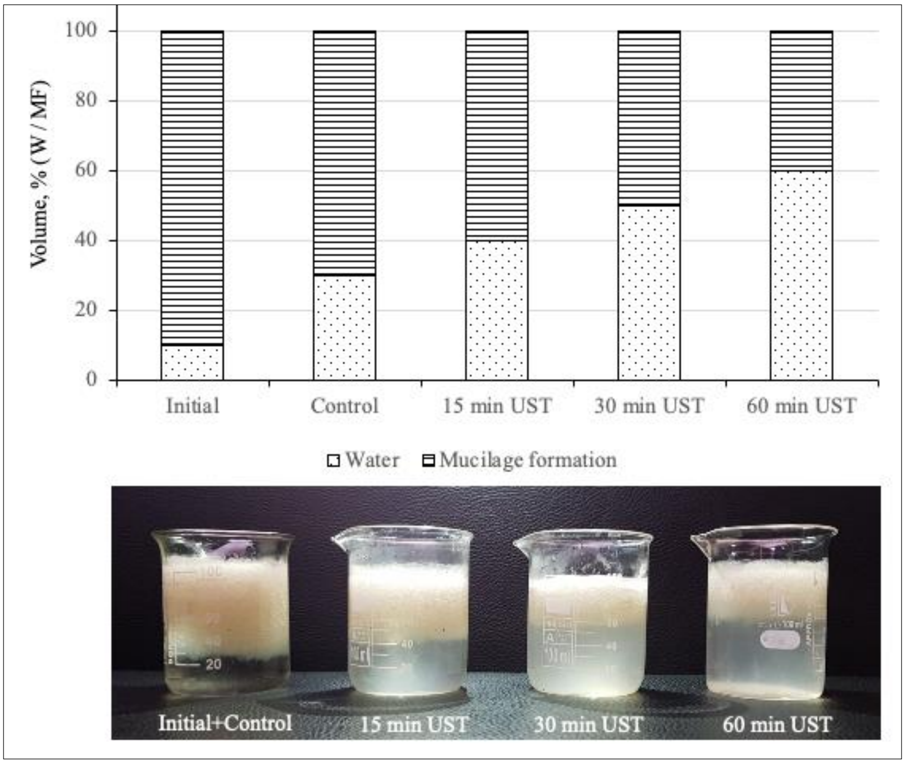

Figure 3. Volumetric proportion of water to mucilage ratio after room-storage (22℃) for 24h

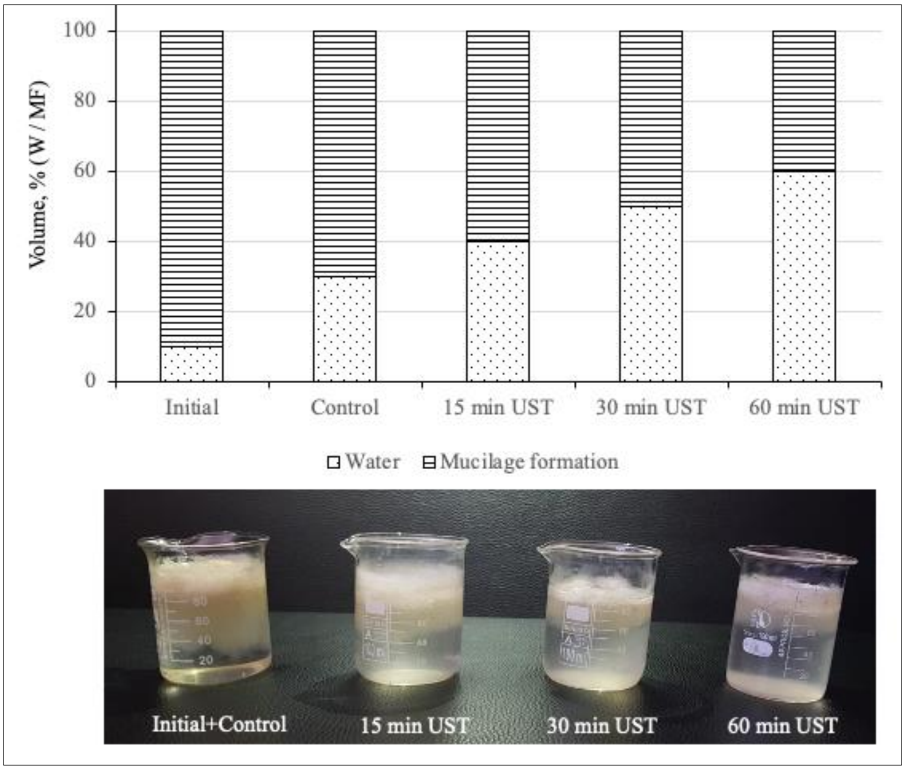

Figure 4. Volumetric proportion of water to mucilage ratio after room-storage (22℃) for 96h

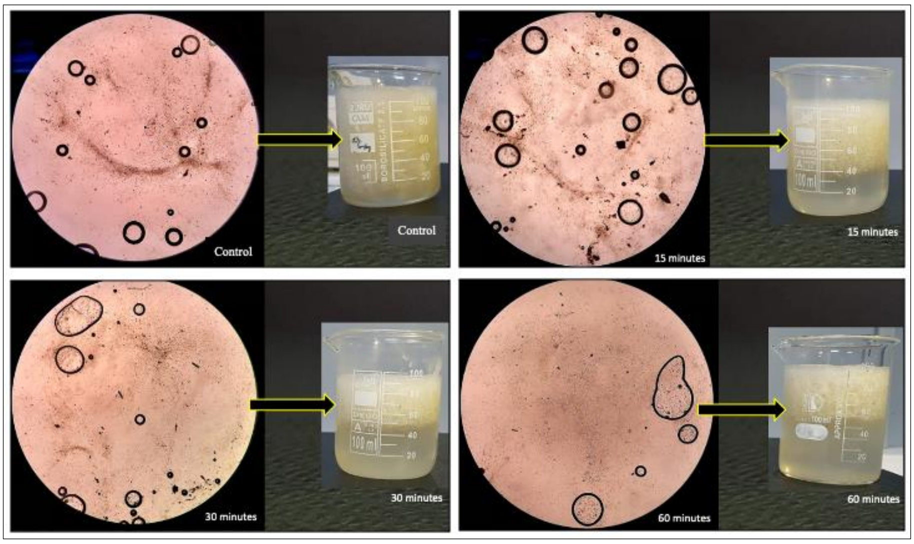

Figure 5. Microscopic image captures of mucilage structure exposed to ultrasound treatment (40 kHz frequency) for 15, 30, 60 min.

The image captures of the control group without ultrasound treatment after 60 min exposure time were similar to the initial structure before the acoustic trial. In the beginning, mucilage formation showed a clustered polymeric structure. In contrast, this clumping formation turned into dispersed particles after 60 min of treatment, a possible sign for the dispersal of aggregates or degradation process of the colloidal structure. Microscopic image captures of the mucilage structure stored for 96 h in room ambience (22 °C) after sonication for 15, 30, and 60 min are shown in Fig. 6.

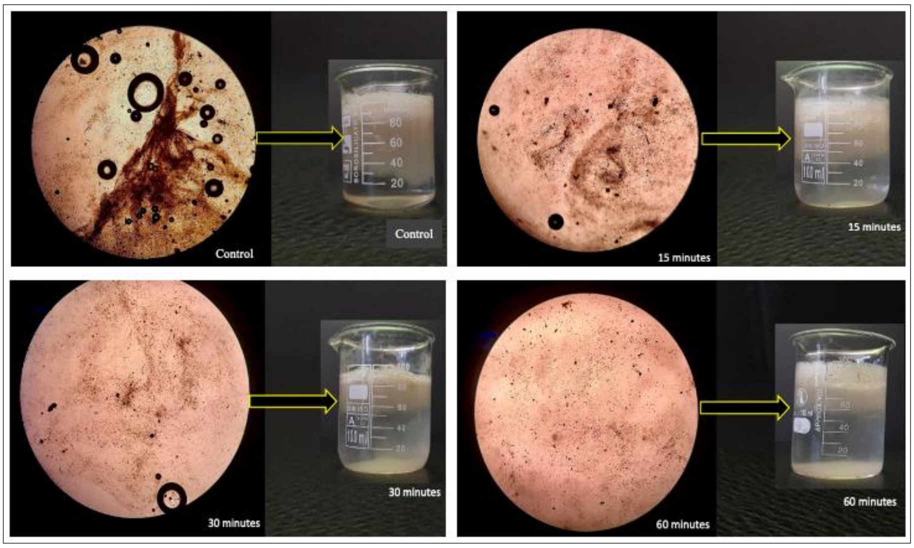

Figure 6. Microscopic image captures of mucilage structure stored in room temperature (22°C) for 96 h after sonication (40 kHz frequency) for 15, 30, and 60 min

Room temperature storage (22°C) for 96 h did not influence the mucilage structures in all treatment groups of exposure durations. Also, mucilage samples in the control group without acoustic treatment showed the same clustered polymeric structure after 96 h storage. The application time was the main factor influencing mucilage structure with gradual dispersal of particles with increasing application time. The highest dispersal of the polymeric formation with scattered particles was noted after 60 min of sonic application.

4. Discussion

The preliminary study results suggest that an ultrasonic treatment system operating at 40 kHz might be effective for dispersing colloidal mucilage formation at the water surface. Clear evidence was found that the dispersal of mucilage structure via sonication is time-dependent, and based on the acoustic trial, it was determined that 60 minutes of exposure duration could be enough for nearly 50% destruction of the structure.

After a comprehensive literature search, we concluded that no published reports are available on the use of acoustic energy on mucilage dispersal. Therefore, the findings in this study were compared with reports focused on the effects of acoustics on several other biofouling organisms and microbial cell properties of bacteria and plankton. The effectiveness of acoustic energy has been investigated on the mortality rates of several organisms suspended in water. In an earlier investigation using ultrasound of three different frequencies (19.5, 28, and 50 kHz) [47], 19.5 kHz frequency was reported as most effective for the destruction of barnacle settlements with 50% mortality, a ratio that was achieved in the challenge with mucilage formation after 60 min treatment in this study with a higher frequency level of 40 kHz. Similarly, another study reported 23 kHz as the most effective frequency on barnacle settlement inhibition with resonant ultrasound frequencies of 23, 63, and 102 kHz [48]. Microbial inactivation [30], and detrimental impacts on bacterial growth through ultrasonic treatment (> 20 kHz) were reported earlier [31, 32], and removal of biofouling organisms was succeeded with 20 kHz frequency [37]. Settlement inhibition of biofouling organisms exposed to 17-30 kHz frequency [36], or 23 kHz frequency [38, 48] has been reported, underlining enhanced settlement inhibition with increasing acoustic exposure time [49], supporting our findings for the time-dependent increase of mucilage dispersal in this study. No biofouling of barnacles, worms, or mussels was observed when ultrasound of 24 kHz was used [26]. In a comprehensive investigation [21], it was reported that sonication between 20 and 42 kHz can be effectively used as a destructive measure in biofouling control of mussel infestation based on earlier reports [39, 40]. Induced mortality rate in algae (35%), or in cysts (55%), larvae (100%) and adult brine shrimp (85%) after exposure to sonication at 1.4 kHz for 20 min was reported earlier [50]. Destructive impacts of ultrasound on survival rate were also reported in earlier investigations [51, 52]. Further, high-power ultrasonic pulses between 28 kHz and 200 kHz were studied, and researchers underlined that higher frequency resulted in higher mortality in barnacle larvae [51]. Another study indicated that 20 kHz pulverized barnacle larvae within 45 sec [52]. Sonication at 19 kHz was investigated on mortality rates of bacteria, phytoplankton such as dinoflagellate, diatom, cyanobacterium, which are reported as the main reason for mucilage formation [1, 6-9] and zooplankton such as brine shrimp, cladoceran, and rotifers in ballast water treatment [53]. The authors reported that ultrasonic treatment efficiency is time-dependent and can show variations with the size of the organisms. Further, it was concluded that an ultrasonic treatment alone for ballast water operating at 19–20 kHz could be efficient to destroy planktonic organisms with sizes over 100 μm. However, smaller sized planktonic organisms such as phytoplankton and bacteria may need additional treatment methods accompanied by advanced oxidation techniques [53]. This finding shows a correlation between sonication efficiency and the size of the target organism.

Considering an unbalanced equilibrium between degradation and aggregation reactions of organic matter through prokaryotic bacteria by enzymatic activities in anoxic conditions, altogether forming the mucilage structure [10, 15-17], oxidation techniques can be coupled with ultrasonic treatment to reduce treatment time and efficiency of ultrasound on mucilage dispersal as suggested earlier [53].

Mucilage is formed due to an altered mechanism from the aggregation of organic matter if the degradation process is slower than polymerization and assembly [10]. In the present study, the exo-polymeric structure of mucilage flocs at water surface changed into rather scattered forms after acoustic treatment of sonication at 40 kHz frequency for 60 min. This could be attributed to the dispersal and separation of large molecules combined with aggregates of small molecules forming the exo-polymeric structure of mucilage [54]. However, further investigations are necessary to clarify the structural change of exo-polymeric flocculation better. Room temperature storage (22℃) for four days (96 h) did not cause any further alteration in the mucilage structure with the same dispersal characteristic of the samples before storage, showing that mucilage exposed to sonication did not re-flocculate after storage in ambient conditions.

Ultrasound applications have been used to inactivate and prevent the proliferation of bacterial cells in foods [55-57], to prevent bacterial growth on various surfaces [30-32] or reduce the effects of planktonic bacteria such as Cyanobacteria, *Escherichia coli* in water environment [58-59]. Further, it has been stated that irreversible lethal effects can be created on both *Escherichia coli* and *Staphylococcus aureus* bacteria through a frequency of 20 kHz exposure for 20 min [60] Liao et al., 2018). Another study underlined that ultrasonic waves (20 kHz) alone could not destroy the yeast's cells, but the damage on cells was sensitive to temperature increase [43]. The resistance of *S. cerevisiae* cells to ultrasound (20 kHz) at 35, 45 and 55℃ in Sabouraud broth was investigated [44], where the authors observed no structural differences among sonicated cells and heat-treated cells. However, the sonication at 45℃ showed cell damage with leakage of intracellular contents. This indicates that the effectiveness of ultrasound in combination with heat treatment within a specific temperature range, could be stronger than ultrasound or heat treatment alone [30]. The expansion of the exo-polymeric structures comprised by the combination of high molecular compounds (polysaccharides, proteins, lipo-polysaccharides, glycolipids, lipids, peptides and nucleic acids) [61-63] can change according to the availability of nutrients [64], and temperature conditions [45, 46]. The development of biofouling might depend on a variety of environmental factors such as salinity, temperature, conductivity, pH, dissolved oxygen, organic material content, hydrodynamic conditions, currents, light, depth, and distance from the shore, or a combination of all these factors [65]. Therefore, temperature range and other water quality parameters need considerations in future investigations on acoustic treatment in the challenge with mucilage formation.

Further, the disruptive effect of ultrasound with a particular frequency depends on types of microorganisms and is likely to be species-specific, with combined influences of environmental conditions. In an investigation on the effects of ultrasonic waves on *Chlamydomonas concordia* and *Dunaliella salina* at frequencies of 20, 585, 864 and 1146 kHz with different acoustic powers, a reduction in algal numbers depending on both ultrasonic frequency and power was reported along with strong relation to the mechanical properties of the cells [66]. Ultrasonic treatment resulted in lethal damage to *Enterobacter aerogenes* and *Bacillus subtilis*, while Staphylococcus spp. was not remarkably affected when treated with ultrasound at 20 kHz frequency [67]. Further, the authors stated that the resistance to the ultrasonic treatment may depend on the cell wall properties of the bacteria and that microorganisms with "thick" or "thin" cell walls may be more resistant to ultrasounds [67]. From this point of view, it can be underlined that ultrasonic frequency may influence different microorganisms in different ways depending on their physical and biological properties. Additionally, higher sensitivity of cells with more complex structures to ultrasonic sounds than cells with relatively small structures was reported earlier [68, 69]. The impacts of ultrasonic treatment on bacteria may also differ according to the intensity and length of sound frequency exposure [32, 63, 70]. Even though several published reports have remarkable information about the effectiveness of ultrasonic sound frequency on the deactivation of bacteria, the relation between the inactivity impacts of sounds Physico-chemical structure of bacteria has not been clearly assessed [32], and still needs further investigation.

As a result, our findings in the present study indicate that the effect of ultrasound on mucilage dispersal is time-dependent and an ultrasound wave with 40 kHz frequency for 60 minutes of application could be enough for about 50% dispersal of the complex matrix of aggregates coupled with bacteria proliferation towards branched molecules of the tree-like mucilage formation.

5. Conclusion

Based on the findings of the preliminary study, it was evident that ultrasound wave treatment with 40 kHz frequency affected mucilage aggregation with dispersal of aggregates forming the mucilage structure. This is the first attempt to investigate the destructive effectiveness of acoustic energy on mucilage formation related to exposure duration. Information regarding the effectiveness of acoustic energy on the dispersal of aggregates forming the mucilage may provide useful implications as a rapid response in the challenge with mucilage formation in nearshore marine ecosystems. Investigations in more detail are encouraged regarding changes of the characteristics of exo-polymeric substances in the mucilage flocculation for a wider understanding of mechanisms involved in mucilage disruption, especially the inactivation process of biological and enzymatic features of microorganisms exposed to acoustic energy. Additionally, introducing acoustic energy in the aquatic environment represents anthropogenic noise, a source of underwater sound pollution that potentially might cause unwanted side-effects on marine life. Therefore, further research on acoustic energy with frequency ranges effective to mucilage but harmless to non-target aquatic species without detrimental side effects is still an open area for further investigations.

Acknowledgements

This study was carried out within the scope of the "Struggle Mobilization" against Mucilage, initiated by the Minister of Environment and Urbanization in Turkey, within the framework of the Marmara Sea Action Plan. Prof. Dr. Sedat MURAT, President of Canakkale Onsekiz Mart University is acknowledged for his valuable support to the laboratories of Aquaculture and Marine Technology Engineering and at the Faculty of Marine Sciences and Technology.

References

[1] Mingazzini, M., Colombo, S., Ferrari, G.M., "Application of spectrofluorimetric techniques to the study of marine mucilages in the Adriatic Sea: preliminary results", Sci Total Environ 165 (1995) : 133-144.

[2] MacKenzie, L., Sims, I.M., Beuzenberg, V., Gillespie, P., "Mass accumulation of mucilage caused by dinoflagellate polysaccharide exudates in Tasmanian Bay, New Zealand", Harmful Algae 1 (2002) : 69-83.

[3] Pompei, M., Mazziotti, C., Guerrini, F., Cangini, M., Pigozzi, S., Benzi, M., Palamidesi, S., Boni, L., Pistocchi, R., "Correlation between the presence of *Gonyaulax fragilis* (Dinophyceae) and mucilage phenomena of the Emilia-Romagna coast (northern Adriatic Sea)", Harmful Algae 2(4) (2003) : 301-316.

[4] Metaxatos, A., Panagiotopoulos, C., Ignatiades, L., "Monosaccharide and aminoacid composition of mucilage material produced from a mixture of four phytoplanktonic taxa", J Exp Mar Biol Ecol 294 (2003) : 203-217.
[5] Nikolaidis, G., Aligizaki, K., Koukaras, K., Moschandreou, K., "Mucilage phenomena in North Aegean Sea, Greece: another harmful effect of dinoflagellates?", 12th International Conference on Harmful Algae, Copenhagen, Denmark, 4-8 September 2006.
[6] Degobbis, D., Fonda, Umani, S., Franco, P., Malej, A., Precali, R., Smodlaka, N., "Changes in the northern Adriatic ecosystem and hypertrophic appearance of gelatinous aggregates", Sci Total Environ 165 (1995) : 43-58.
[7] Balkis, N., "Seasonal variations in the phytoplankton and nutrient dynamics in the neritic water of Büyükçekmece Bay, Sea of Marmara", J Plankton Res 25 (2003) : 703-717.
[8] Najdek, M., Blazina, M., Djakovac, T., Kraus, R., "The role of the diatom *Cylindrotheca closterium* in a mucilage event in the northern Adriatic Sea: coupling with high salinity water intrusions", J Plankton Res 27 (2005) : 851-862.
[9] Tüfekçi, V., Balkıs, N., Polat Beken, Ç., Ediger, D., Mantıkçı, M., "Phytoplankton composition and environmental conditions of a mucilage event in the Sea of Marmara", Turk J Biol 34 (2010) : 199-210.
[10] Mecozzi, M., Pietroletti, M., Conti, M.E., "The complex mechanisms of marine mucilage formation by spectroscopic investigation of the structural characteristics of natural and synthetic mucilage samples", Mar Chem 112(1-2) (2008) : 38-52.
[11] Simon, M., Grossart, H.P., Schweitzer, B., Ploug, H., "Microbial ecology of organic aggregates in aquatic ecosystems", Aquat Microb Ecol 28 (2002) : 175-211.
[12] Shanks, A.L., Trent, J.D., "Marine snow: microscale nutrient patches", Limnol Oceanogr 24 (1979) : 850-854.
[13] Aldredge, A.L., Granata, T.C., Gotschalk, C.C., Dickey, T.D., "The physical strength of marine snow and its implications for particle disaggregation in the ocean", Limnol Oceanogr 35(7) (1990) : 1415-1428.
[14] Tansel, B., "Morphology, composition and aggregation mechanisms of soft bioflocs in marine snow and activated sludge: A comparative review", J Environ Manage 205 (2018) : 231-243.
[15] Precali, R., Giani, M., Marini, M., Grilli, F., Ferrari, C.R., Pecar, O., Paschini, E., "Mucilaginous aggregates in the Northern Adriatic in the period 1999–2002: typology and distribution", Sci Total Environ 353 (2005) : 10-23.
[16] Muller-Niklas, G., Schuster, S., Kaltenbock, E., Herndl, G.J., "Organic content and bacterial metabolism in amorphous aggregations of the Northern Adriatic Sea", Limnol Oceanogr 39 (1994) : 58–68.
[17] Obernosterer, I., Herndl, G.J., "Phytoplankton extracellular release and bacterial growth: Dependence on the inorganic N:P ratio", Mar Ecol Prog Ser 116(1-3) (1995) : 247-257.
[18] Herndl, G.J., Peduzzi, P., "Ecology of amorphous aggregations (marine snow) in the Northern Adriatic Sea: I general considerations", PSZNI Mar Ecol 9 (1988) : 79-90.
[19] Herndl, G.J., "Marine snow in northern Adriatic Sea: possible causes and consequences for a shallow ecosystem", Mar Microb Food Webs 6 (1992) : 149-172.
[20] Fogg, G.E., "Some speculations on the nature of the pelagic mucilage community of the northern Adriatic Sea", Sci Total Environ 165 (1995) : 59-63.

[21] Donskoy, D.M., "The use of acoustic, vibrational, and hydrodynamic techniques to control zebra mussel infestation. Technical Report, New Jersey Marine Sciences Consortium. Technical Report SIT-DL-96-9-2736. Davidson Laboratory Project 5437/62 (1996) New Jersey. https://rucore.libraries.rutgers.edu/rutgers-lib/46935/PDF/1/play/ (accessed 21 Setember 2021).

[22] Tiller, G.W., Gaucher, T.A., Menezes, J.K., Dolat, S.W., "Zebra mussel control using acoustic energy", Proc Am Power Conf 54(1) (1992) Conference: 54. Annual American power conference, Chicago, IL (United States), 13-15 Apr 1992; Journal ID: ISSN 0097-2126. https://www.osti.gov/biblio/7117379-zebra-mussel-control-using-acoustic-energy. (accessed 13 September 2021).

[23] Dai, C., Xiong, F., He, R., Zhang, W., Ma, H., "Effects of low-intensity ultrasound on the growth, cell membrane permeability and ethanol tolerance of *Saccharomyces cerevisiae*" Ultrason Sonochem 36 (2017) : 191-197.

[24] Pulkki, V., McCormack, L., Gonzalez, R., "Superhuman spatial hearing technology for ultrasonic frequencies", Sci Rep 11 (2021) : 11608.

[25] Elpiner, I.E., Feigina, Z.S., "Ultrasound waves in the fight against hydrobionts", Vodosnabzheniye i Sanitarnaya Tech 6 (1957) : 14-16.

[26] Latour, M., Murphy, P., "Application of PVF2 transducers as piezoelectric vibrators for marine fouling prevention", Ferroelectrics 32(1) (1981) : 33-37.

[27] Branscomb, E.S., Rittschof, D., "An investigation of low frequency sound waves as a means of inhibiting barnacle settlement", J Exp Mar Biol Ecol 79(2) (1984) : 149-154.

[28] Fischer, E., Castelli, V., Rodgers, S., Bleile, H., "Technology for Control of Marine Biofouling - A Review", in: Costlaw, J.D., Tipper, R.C. (Eds.), Marine Biodeterioration: An Interdisciplinary Study. Naval Institute Press, Annapolis MD (1984) : 261-300

[29] Choi, C., Scardino, A., Dylejko, P., Fletcher, L., Juniper, R., "The effect of vibration frequency and amplitude on biofouling deterrence", Biofouling 29(2) (2013) : 195-202.

[30] Huang, G., Tang, Y., Sun, L., Xing, H., Ma, H., He, R., "Ultrasonic irradiation of low intensity with a mode of sweeping frequency enhances the membrane permeability and cell growth rate of *Candida tropicalis*", Ultrason Sonochem 37 (2017) : 518-528.

[31] Pitt, W.G., Ross, S.A., "Ultrasound increases the rate of bacterial cell growth", Biotechnol Prog 19(3) (2003) : 1038-1044.

[32] Gao, S., Lewis, G.D., Ashokkumar, M., Hemar, Y., "Inactivation of microorganisms by low-frequency high-power ultrasound: 2. A simple model for the inactivation mechanism", Ultrason Sonochem 21 (2014a) : 454-460.

[33] Kapturowska, A.U., Stolarzewicz, I.A., Krzyczkowska, J., Białecka-Florjanczyk, E., "Studies on the lipolytic activity of sonicated enzymes from *Yarrowia lipolytica*" Ultrason Sonochem 19 (2012) : 186-191.

[34] McDonald, J., Wilkens, S., Stanley, J., Jeffs, A., "Vessel generator noise as a settlement cue for marine biofouling species", Biofouling 30(6) (2014) : 741-749.

[35] Stanley, J.A., Wilkens, S.L., Jeffs, A.G., "Fouling in your own nest: vessel noise increases biofouling", Biofouling 30(7) (2014) : 837-844.

[36] Sheherbakov, P.S., Grigoryan, F.Y., Pogrebnyak, N.V., "Distribution of high frequency vibration in hulls of Krasnograd-class ships equipped with ultrasonic antifouling

protection systems. Transaction", Technical Operations of the Maritime Fleet. Thermochemical Studies. Control of Corrosion and Fouling (1974) : AD 778380

[37] Mazue, G., Viennet, R., Hihn, J., Carpentier, L., Devidal, P., Albaina, I., "Largescale ultrasonic cleaning system: design of a multi-transducer device for boat cleaning (20 kHz)", Ultrason Sonochem 18(4) (2011) : 895-900.

[38] Guo, S., Lee, H., Teo, S., Khoo, B., "Inhibition of barnacle cyprid settlement using low frequency and intensity ultrasound", Biofouling 28(2) (2012) : 131-141.

[39] Sonalysts, Inc. & Aquatic Sciences, Inc. Zebra Mussel deterrence using acoustic energy. Empire State Electric Energy Research Corporation ESEERCO, Research report (1991): EP 90-38.

[40] Sonalysts, Inc. & Aquatic Sciences, Inc, Evaluation of ultrasound for Zebra Mussel mitigation. ESEERCO, Research report (1992) : EP 91-17.

[41] Boelman, S.F., Neilson, F.M., Dardeau, E.A., Cross, Jr.T., "Zebra Mussel (*Dreissena polymorpha*) Control Handbook for Facility Operators, first ed. Zebra Mussel Research Program. Miscellaneous Paper (1997): EL-97-1, U.S. Army Corps of Engineers Washington.https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.1005.6304&rep=rep1&type=pdf (accessed 18 September 2021).

[42] Kowalewski, J.J., Patrick, P.H., Christie, A.E., "Effect of acoustic energy on the zebra mussel (*Dreissena polymorpha*)", in: Nalepa, T.F., Schloesser, D.W. (Eds.), Zebra mussels: Biology, impacts, and control. Lewis Publishers, Boca Raton, FL (1993) : 657-666.

[43] Ciccolini, L., Taillandier, P., Wilhem, A.M., Delmas, H., Strehaiano, P., "Low frequency thermo-ultrasonication of Saccharomyces cerevisiae suspensions: effect of temperature and of ultrasonic power", Chem Eng J 65 (1997) : 145–149.

[44] Guerrero, S., López-Malo, A., Alzamora, S.M., "Effect of ultrasound on the survival of Saccharomyces cerevisiae: influence of temperature, pH and amplitude", Innov Food Sci Emerg Technol 2(1) (2001) : 31-39.

[45] Gandhi, H.P., Ray, R.M., Patel, R.M., "Exopolymer production by Bacillus species", Carbohydr Polym 34 (1997) 323-327.

[46] Underwood, G.J.C., Fietz, S., Papadimitriou, S., Thomas, D.N., Dieckmann, G.S., "Distribution and composition of dissolved extracellular polymeric substances (EPS) in Antarctic Sea ice", Mar Ecol Prog Ser 404 (2010) : 1-19.

[47] Kitamura, H., Takahashi, K., Kanamaru, D., "Inhibitory effect of ultrasonic waves on the larval settlement of the barnacle *Balanus amphitrite* in the laboratory", Mar Fouling 12(1) (1995) : 9-13.

[48] Guo, S., Lee, H.P., Khoo, B.C., "Inhibitory effect of ultrasound on barnacle (Amphibalanus amphitrite) cyprid settlement", J Exp Mar Biol Ecol 409(1) (2011a) : 253-258.

[49] Guo, S., Lee, H., Chaw, K., Miklas, J., Teo, S., Dickinson, G., Birch, W., Khoo, B., "Effect of ultrasound on cyprids and juvenile barnacles", Biofouling 27(2) (2011b) : 185-192.

[50] Gavand, M., McClintock, J., Amsler, C., Peters, R., Angus, R., "Effects of sonication and advanced chemical oxidants on the unicellular green alga *Dunaliella tertiolecta* and cysts,

larvae and adults of the brine shrimp *Artemia salina*: a prospective treatment to eradicate invasive organisms from ballast water", Mar Pollut Bull 54(11) (2007) : 1777-1788.

[51] Suzuki, H., Konno, K., "Basic studies on the antifouling by ultrasonic waves for ship's bottom fouling organisms", J Tokyo Univ Fish 56 (1970) : 31-48.

[52] Seth, N., Chakravarty, P., Khandeparker, L., Anil, A., Pandit, A., "Quantification of the energy required for the destruction of *Balanus amphitrite* larva by ultrasonic treatment", J Mar Biol Assoc UK 90(7) (2010) : 1475-1482.

[53] Holm, E.R., Stamper, D.M., Brizzolara, R.A., Barnes, L., Deamer, N., Burkholder, J.M., "Sonication of bacteria phytoplankton and zooplankton: application to treatment of ballast water", Mar Poll Bull 56(6) (2008) : 1201-1208.

[54] Leppard, G.G., "The characterization of algal and microbial mucilages and their aggregates in aquatic ecosystems", Sci Total Environ 165 (1995) : 103-131.

[55] Ewe, J.A., Abdullah, W.N.W., Bhat, R., Karim, A.A., Liong, M.T., "Enhanced growth of lactobacilli and bioconversion of isoflavones in biotin-supplemented soymilk upon ultrasound-treatment. Ultrason Sonochem 19 (2012) : 160-173.

[56] Guimarães, J.T., Silva, E.K., Alvarenga, V.O., Costa, A.L.R., Cunha, R.L., Santana, A.S., Freitas, M.Q., Meireles, M.A.A, Cruz, A.G., "Physicochemical changes and microbial inactivation after high-intensity ultrasound processing of prebiotic whey beverage applying different ultrasonic power levels", Ultrason Sonochem 44 (2018) : 251-260.

[57] Koua, X., Lia, R., Houa, L., Zhanga, L., Wang, S., "Identifying possible non-thermal effects of radio frequency energy on inactivating food microorganisms", Int J Food Microbiol 269 (2018) 89-97.

[58] Wu, X., Joyce, E.M., Mason, T.J., "The effects of ultrasound on cyanobacteria. Harmful Algae 10 (2011) 738-743.

[59] Zhu, H.X., Cai, X.Z., Shi, A.L., Hu, B., Yan, S.G., "Microbubble-Mediated Ultrasound Enhances the Lethal Effect of Gentamicin on Planktonic *Escherichia coli*", BioMed Res Int 7 (2014).

[60] Liao, X., Li, J., Suo, Y., Chen, S., Ye, Z., Liu, D., Ding, T., "Multiple action sites of ultrasound on *Escherichia coli* and *Staphylococcus aureus*", Food Sci Hum Well 7 (2018) : 102-109.

[61] Branda, S.S., Vik, A., Friedman, L., Kolter, R., "Biofilm: the matrix revisited", Trends Microbiol 13 (2005) : 20-26.

[62] Willis, L.M., Whitfield, C., "Structure, biosynthesis, and function of bacterial capsular polysaccharides synthesized by ABC transporter-dependent pathways", Carbohydr Res 378 (2013) : 35-44.

[63] Di Donato, P., Poli, A., Taurisano, V., Abbamondi, G.R., Nicolaus, B., Tommonaro, G., "Recent advances in the study of marine microbial biofilm: from the involvement of quorum sensing in its production up to biotechnological application of the polysaccharide fractions", J Marine Sci Eng 4 (2016) : 34.

[64] Lee, J.W., Yeoman, S.W.G., Allen, A.L., Deng, F., Gross, R.A., Kaplan, D.L., "Biosynthesis of novel exopolymers by *Aureobasidium pullulans*", Appl Environ Microbiol 65(12) (1999) : 5262-5271.

[65] Delauney, L., Compére, C., Lehaitre, M., "Biofouling protection for marine environmental sensors", Ocean Sci Discuss 6(3) (2009) : 2993-3018. www.ocean-sci-discuss.net/6/2993/2009/

[66] Yamamoto, K., King, P.M., Wu, X., Mason, T.J., Joyce, E.M., "Effect of ultrasonic frequency and power on the disruption of algal cells", Ultrason Sonochem 24 (2015) : 165-171.

[67] Gao, S., Lewis, G.D., Ashokkumar, M., Hemar, Y., "Inactivation of microorganisms by low-frequency high-power ultrasound: 1. Effect of growth phase and capsule properties of the bacteria", Ultrason Sonochem 21 (2014b) : 446-453.

[68] Thacker, J., "An approach to the mechanism of killing of cells in suspension by ultrasound", Biochim Biophys Acta Gen Subj 304(2) (1973) : 240-248.

[69] Nesaratnam, S.T., Wase, D.A.J., Blakebrough, N., "The susceptibility to ultrasonic disintegration of Klebsiella pneumoniae NCTC 418", Eur J Appl Microbiol Biotechnol 15(1) (1982) : 56-58.

[70] Wietzikoski Lovato, E.C., Velasquez, P.A.G., Oliveira, C.S.O., Baruffi, C., Anghinoni, T., Machado, R.C., Lívero, F.A.R., Wietzikoski Sato, S., Martins, L.A., "High frequency equipment promotes antibacterial effects dependent on intensity and exposure time", Clin Cosmet Investig Dermatol 11 (2018) : 131-135.