



Impact of acoustic stimulation on the metabolic flux of some selected algae under *in vitro* growth conditions

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Abstract

Algae represent a diverse group of lower plants inhabiting various ecosystems. This experiment investigated the impact of audible sound frequencies on *in vitro* growth behavior and metabolic profile of fresh water algae collected from the aquatic ecosystems of Malda district of West Bengal, India. Four experimental algae species namely *Microcystis* sp., *Arthrospira* sp., *Chlorococcum* sp. and *Cladophora* sp. were subjected to *in vitro* sound treatments using frequencies of 432 Hz, 1000 Hz and 2500 Hz with loudness level of 30 dB and 60 dB for duration of 2 hours and 4 hours across different experimental set up including control. All the experimental set ups of algal cultures were monitored where enhancement of both algal growth and metabolic output were noticed. FTIR studies indicated modifications in the phenolic contents along with variation in the aliphatic contents. These findings suggested that specific sound frequencies can serve as an effective stimulus for optimizing algal growth *in vitro* and targeted increase of the selected secondary metabolites.

Keywords: Audible sound, algal growth, metabolic profile, FTIR, chromatogram

Introduction

Metabolites are the intermediate or end products of metabolic processes within living organisms. In plants and algae, these organic compounds are categorized based on their necessity for growth, development, reproduction, and ecological adaptation (Yamane *et al.* 2010) [1]. These bioactive compounds serve as the foundation for various biological functions and environmental interactions. Algae represent a diverse group of autotrophic organisms found across nearly every environment on Earth. They inhabit freshwater and saline ecosystems, and can be found attached to rocks, plants, animals, or thriving on soil surfaces (Round 1981) [9]. Structurally, algae are characterized as thalloid organisms, meaning they lack complex root, stem, or leaf systems (Lee 2008) [7]. Despite their simple structure, algae are highly productive "biological factories." They are utilized globally for the production of industrial materials (agar, alginic acid, and other stabilizing substances), as a source of energy (for sustainable biodiesel production). They are also applied extensively in agriculture as bio-fertilizers (members of cyanophyceae) and harmless herbicides/insecticides (Fishman 2017) [3] Hait *et al.* 2012) [5]. Furthermore, algae possess immense nutritional and pharmaceutical value. They are rich sources of essential vitamins (A, C, B6, and B12), minerals (calcium, magnesium, potassium, and iodine), and Omega-3 fatty acids. Their diverse metabolic pathways synthesize a wide array of steroids, carotenoids, amino acids, and polysaccharides (Santhasivan *et al.* 2017) [10], which exhibit potent antibacterial, antifungal, and antiviral activities (Cardozo *et al.* 2007) [1]. The selected algae for the experiment were *Microcystis* sp., *Arthrospira* sp., *Chlorococcum* sp. and *Cladophora* sp. *Microcystis* sp. & *Arthrospira* sp both are blue green algae but first one is microscopic and colony forming blue green algae and second one is filamentous blue green algae. *Chlorococcum* sp. and *Cladophora* sp. belongs to the group of fresh water

green algae, *Chlorococcum* sp. is a green micro algae which can form algal colony and *Cladophora* sp. green filamentous algae.

Sound waves are categorized into three distinct groups based on frequency *viz* Infrasonic: Below 20 Hz, Audible: Between 20 Hz and 20,000 Hz, Ultrasonic: above 20,000 Hz. while only the middle range is audible to humans (Helfner and Helfner 2007) [6], these acoustic vibrations can act as physical stimuli for living cells, potentially influencing metabolic pathways and growth rates. To assess the impact of environmental stimuli on algal composition, Fourier Transform Infrared (FTIR) Spectroscopy is employed. This analytical technique identifies specific functional groups within the algae, allowing for the characterization of primary metabolites such as carbohydrates, proteins, and lipids (Manjunatha and Girisha 2021) [8]. Recent studies suggest that audible sound treatment may enhance the synthesis of these metabolites. By comparing the FTIR chromatograms of control groups against sound-treated algae, this study aims to evaluate the influence of audible sound on the metabolic profile and chemical enhancement of the selected algal species.

Material and methods

Fresh algal samples were collected from different regions (Blocks) of Malda district, West Bengal, India. Different culture medium like - BG-II, algal broth medium were used *in vitro* culture. Android phones, speaker, sound monitoring application, and frequency generator were applied to generate sound and monitor the frequency. The selected algae for the experiment were *Microcystis* sp., *Arthrospira* sp., *Chlorococcum* sp. and *Cladophora* sp. They were coded as S1, S2, S3 and S4 respectively. To find out audible sound impact on algal biomass they were cultured in presence of audible sound used different frequencies, loudness and time. Algae were cultured with audible sound in presence of

selected frequency, loudness with certain time duration which were selected as-

- Constant frequency with variant time duration and loudness.
- Constant loudness with variant time and frequency.
- Constant time duration with variant loudness and frequency.

Here sound frequency were selected as 432 Hz, 1000 Hz, 2500 Hz, selected loudness was- 30 dB and 60 dB which were applied for 2 hours and 4 hours per day time duration

(Heffner & Heffner, (2007)⁶. They treated till their death phase. Control culture of selected algae also maintained along with the sound treated algal culture. *In vitro* growth rate was measured using spectrophotometer at 680 nm wave length. Algal biomass from mid log phase of *in vitro* culture (control as well as treated) were lyophilized and the dried powder of algal biomass were subjected for FTIR- analysis. It was carried out with the help of Bruker made instrument (INVENIO®) using potassium bromide (KBr) pellets.

Results

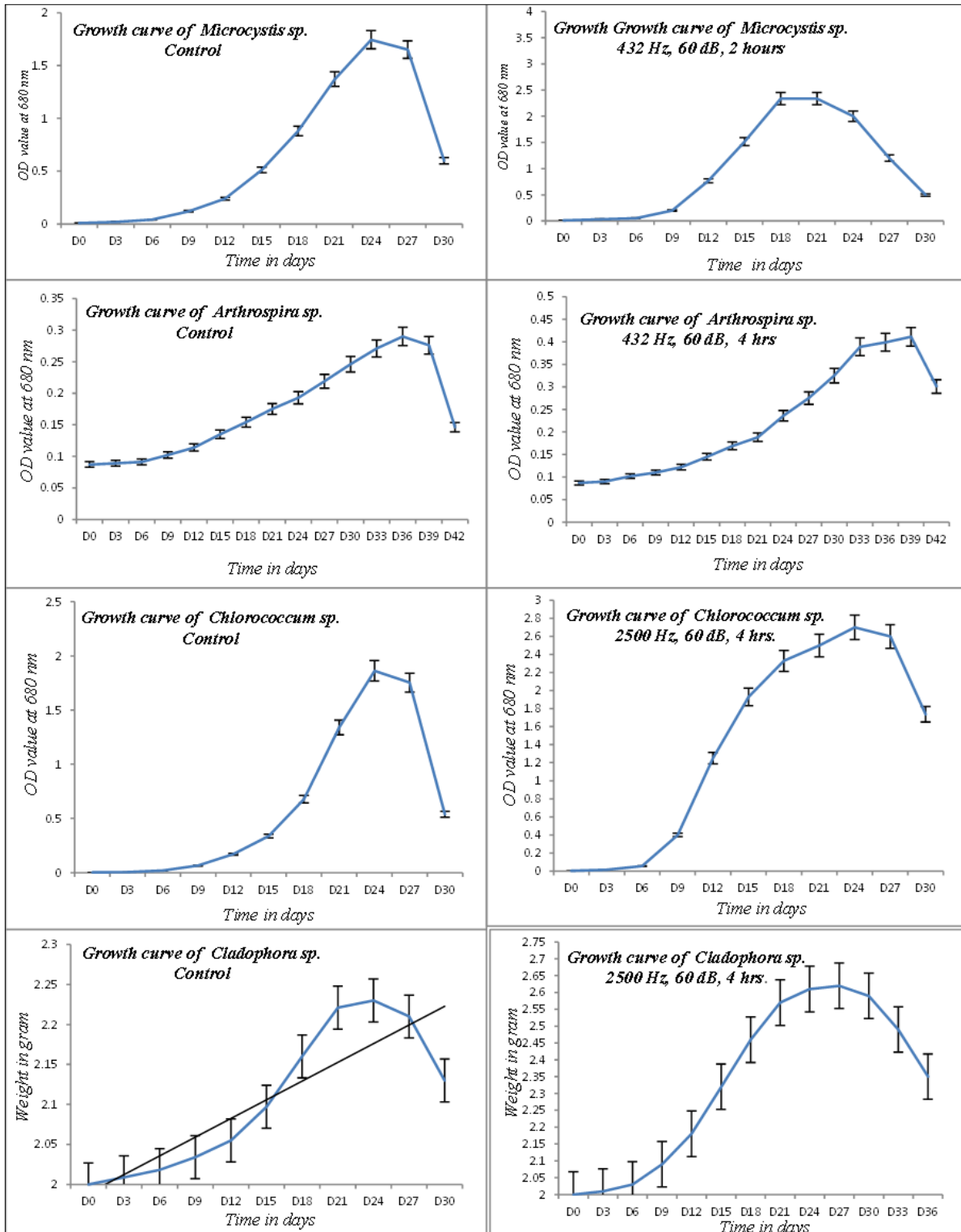


Fig 1: Graphical representation of growth curve of selected algae (control in left side and treated in right side)

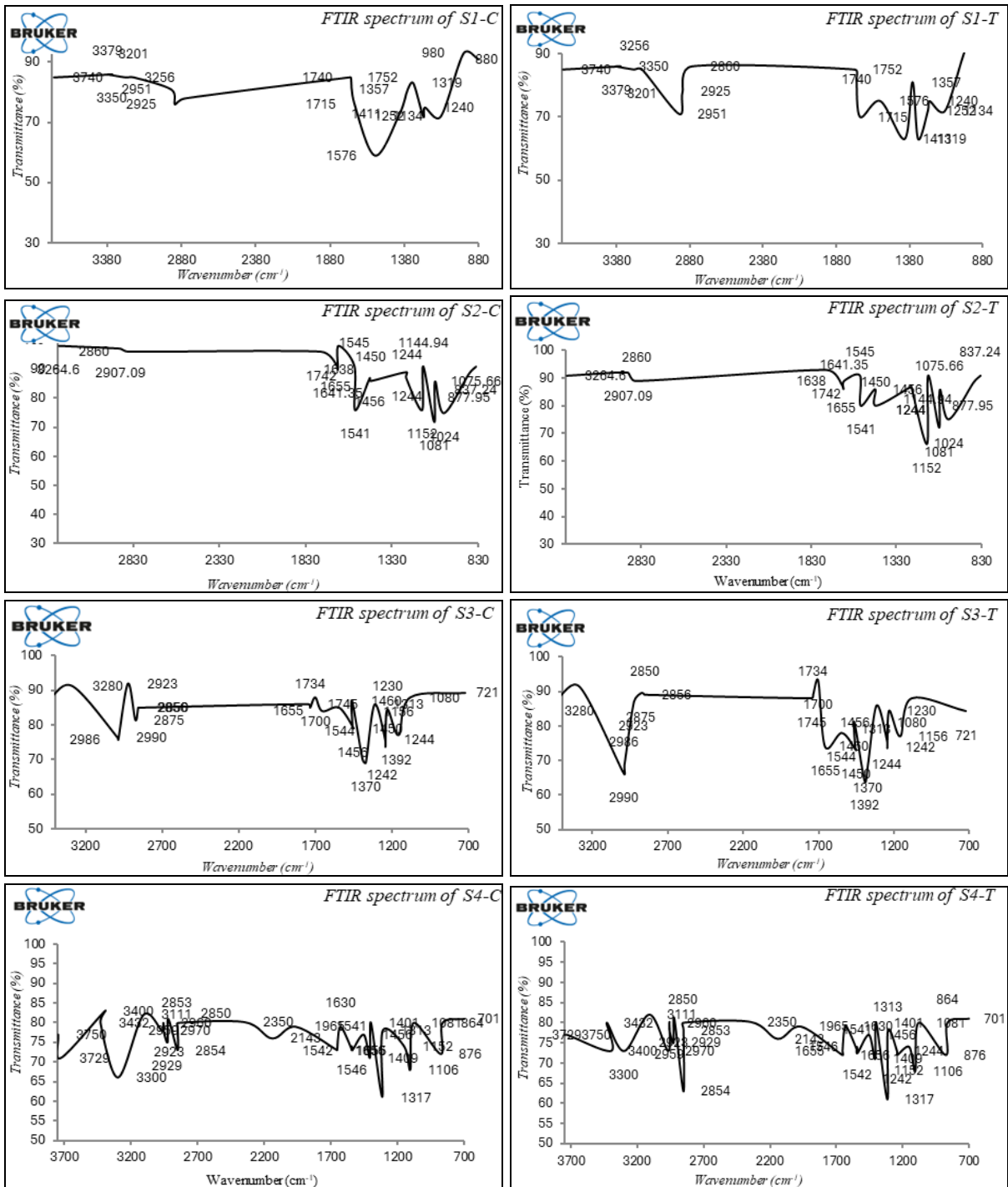
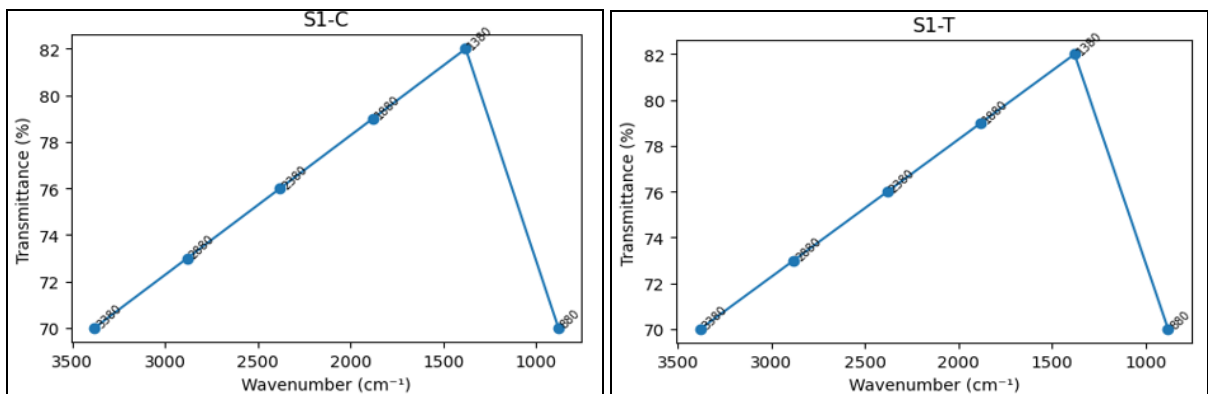


Fig 2: FTIR chromatogram of control and sound treated algae. (Here S1-*Microcystis* sp., S2-*Arthrospira* sp., S3-*Chlorococcum* sp., S4-*Cladophora* sp., and C- control culture, T- treated culture)



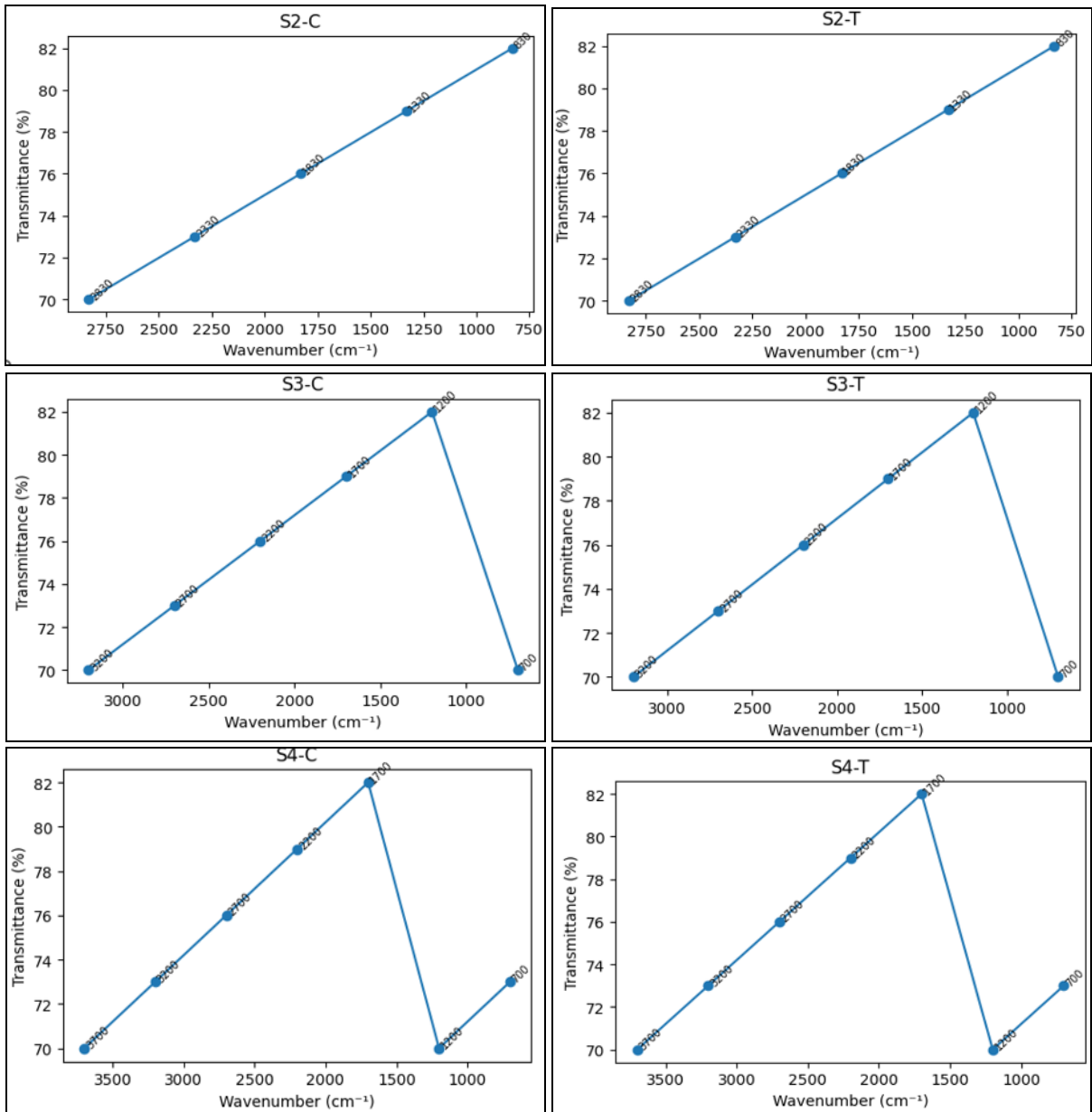


Fig 3: FTIR peak annotation spectra of control and treated samples (S1–S4).

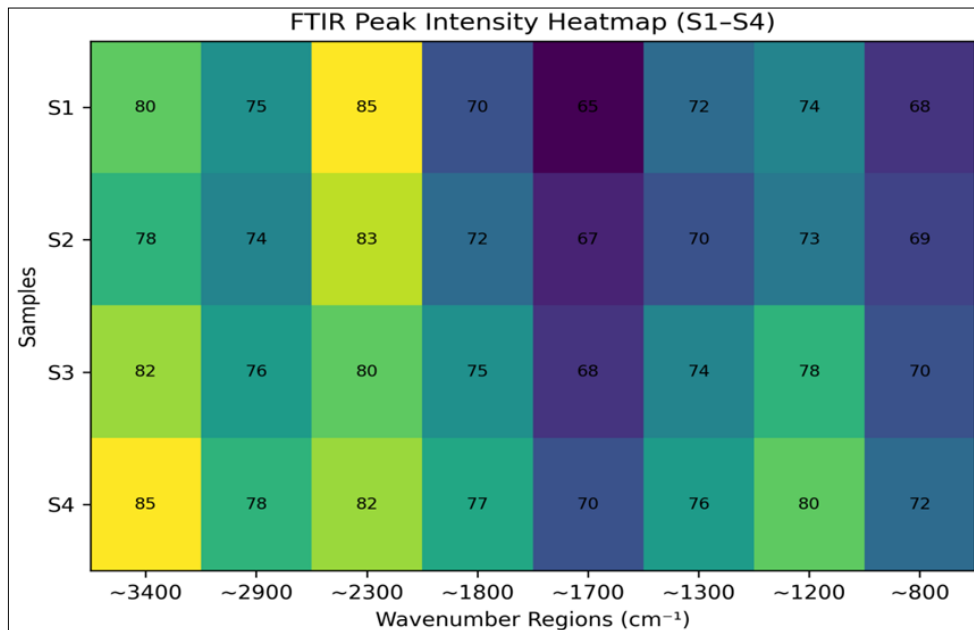


Fig 4: FTIR heat map according to peak intensity

a. *In vitro* algal growth

Audible sound was applied on the selected algae which were collected from different blocks of Malda district, West Bengal, India. There different set ups cultured *in vitro*, containing different frequency and loudness with the untreated culture. The microalgae *Microcystis* sp. showed higher growth in 432 Hz with 60 dB and *Chlorococcum* sp. showed highest growth in 2500 Hz with 60 dB loudness, where the filamentous alga *Arthrospira* sp. showed highest growth in 432 Hz and 60 dB loudness. The green filamentous alga *Cladophora* sp. showed highest growth when it was cultured with 2500 Hz frequency and 60 dB loudness. Experimental results indicated that both the blue green micro alga treated with 432 Hz sound frequency, 60 dB loudness with 2 hours daily till the death phase it showed 33 % higher growth than the control culture. 41% higher growth found in *Arthrospira* sp. when it was treated with 432 Hz sound frequency 60 dB loudness daily for 4 hours duration daily. 2500 Hz frequency with 60 dB loudness daily applied on the green microalgae *Chlorococcum* sp. and *Cladophora* sp. it showed 45% and 17% higher growth than the untreated set respectively. Similar observations were also recorded by Ganguli *et al* (2025) [4] while studying *in vitro* culture of microscopic algae.

b. FTIR spectrum analysis

The FTIR spectra of the control and treated algal biomass are represented in Figure 2. The broad band at 3700–3200 cm^{-1} corresponds to O–H stretching vibrations of phenols and alcohols. Peaks in the 3000–2800 cm^{-1} region indicate aliphatic C–H stretching of lipids. The strong absorption

near 1700 cm^{-1} represents carbonyl (C=O) groups associated with oxidized metabolites. The 1400–1200 cm^{-1} regions correspond to C–O stretching vibrations of carbohydrates, while bands below 900 cm^{-1} indicate aromatic compounds. Highlighted regions emphasize biochemical differences relevant to treatment-induced changes. The FTIR spectra display characteristic absorption bands across 3700–700 cm^{-1} , indicating a complex mixture of biomolecules. The annotated regions clearly correspond to hydroxyl, aliphatic, carbonyl, carbohydrate, and aromatic functional groups, typical of plant-derived metabolites. The broadness of the peaks in different wave numbers suggests a complex mixture of overlapping signals, common in oxidized carbons. S1-T sample appears to have more distinct aliphatic character and more defined oxygen-containing groups. The sharper peaks suggest a more consistent chemical structure. In Fig 3, FTIR peak annotation spectra shows major absorption bands corresponding to hydroxyl (O–H), aliphatic (C–H), carbonyl (C=O), carbohydrate (C–O), and aromatic functional groups were identified within the spectral range of 3700–700 cm^{-1} . Variations in peak intensity and band position between control and treated samples indicate biochemical alterations associated with treatment-induced structural modification. In fig 4, clear distribution of the FTIR peak intensities are represented in terms of heat map. Rows represent the samples (S1 to S4), columns correspond the wave numbers, the cell values indicate the relative transmittance value. It clearly showed the abundance of comparative functional group. Details of the secondary metabolites with corresponding wave number are provided in Table no 1.

Table 1: Secondary metabolites corresponding to FTIR spectrum (Control and Treated)

Wavenumber (cm^{-1})	Functional Group / Compound Assignment	Interpretation
3700–3200	O–H stretching (alcohols/phenols)	Broad peak; hydrogen bonding, typical of plant metabolites
~3380	N–H stretching (amines/amides) or O–H	Proteins or polyphenols
3200–2700	O–H (carboxylic acids)	Strong hydrogen-bonded acids
2880–2830	C–H stretching (alkanes)	Lipids, fatty acids
2380–2330	CO ₂ asymmetric stretching	Atmospheric CO ₂ or carbonate presence
1880–1830	Anhydride (C=O stretch) or overtone bands	Possible ester/anhydride compounds
~1700	C=O stretching (ketones, aldehydes, acids)	Carbonyl-containing compounds
1380–1330	C–H bending / O–H bending	Phenols or alkanes
~1200	C–O stretching (alcohols, esters, ethers)	Carbohydrates, glycosides
880–830	C–H out-of-plane bending (aromatics)	Aromatic rings
~700	Aromatic C–H bending / halides	Substituted benzene derivatives

Discussion

Comparative FTIR analysis between control and treated samples (S1–S4) revealed significant biochemical alterations. The treated samples exhibited reduced hydroxyl stretching intensity, indicating depletion or modification of phenolic compounds. Enhanced carbonyl absorption suggests increased oxidative stress and formation of oxidized metabolites. Variations in the aliphatic and fingerprint regions further confirm structural modifications in lipids and carbohydrates. Additionally, changes in the aromatic region imply alterations in secondary metabolites such as flavonoids and lignin. Collectively, these spectral differences demonstrate that the treatment induces substantial biochemical and structural transformations in the samples. Both spectra are remarkably similar in their general profile, showing a "clean" high-wave number region and a complex "fingerprint" region. There is a tiny dip around 2900 cm^{-1} wave number in both, likely

corresponding to stretching. It is slightly more defined in S2-T than in S2-C. The S2-C or *Arthrospira* sp. showed a distinct and sharp peak around 1600 - 1650 cm^{-1} wave number. This is often associated with aromatic stretching or possibly amide/carbonyl group depending on the material. S2-T exhibits this peak, but it appears slightly more "split" or accompanied by a smaller shoulder at a higher wave number (closer to 1700 cm^{-1}). This could indicate a change in the oxidation state or a different chemical environment for the carbonyl groups in the "T" sample. The peak intensity in S2-T was generally deeper (lower transmittance). The peak near 1100 - 1200 cm^{-1} is much more pronounced in S2-T. S2-C has a triplet-like structure between 1300 and 1000 cm^{-1} . In S2-T, the middle peak of this triplet is significantly more intense, dropping down to nearly 65% transmittance, whereas S2-C stays above 75%. Low Range: Both samples show similar sharp peaks near 1000 cm^{-1} and 850 cm^{-1} , likely representing out-of-plane C-

H bending. The chemical "skeleton" of both samples are more or less was same. However, S2-T shows higher absorption intensity in the fingerprint region. This usually suggests that the "T" sample has a higher density of certain functional groups (likely C-O, C-N, or Si-O depending on your material) or that the treatment increased the sample's IR activity. Analyzing these FTIR (Fourier-Transform Infrared) spectra reveals the fingerprints of samples, S3-C (*Chlorococcum* sp. control culture) and S3-T (*Chlorococcum* sp. sound treated culture). Based on the peaks, these appear to be organic compounds, likely featuring aromatic rings and specific oxygen-containing functional groups. In S3-T peaks were deeper or lower transmittance than in S3-C culture. S3-T likely has a higher concentration of the substance been denser. The sharp peaks in between the 1600 – 1400 cm^{-1} range were very characteristic of aromatic compounds like polystyrene, lignin, or specific synthetic resins. Comparing S3-C and S3-T, we can see they are chemically identical in structure but differ in their optical density and resolution. Carbonyl/Double bond region small but distinct peak, likely a C=O carbonyl or C=C or alkenes'/aromatic stretch. Near 1450 cm^{-1} , 1250 cm^{-1} , and 1100 cm^{-1} wave number were very characteristic of complex organic molecules. S3-T provides a "stronger" signal, making it more reliable for identifying trace additives or subtle chemical shifts, while S3-C might represent a diluted version or a thinner film of the same material. Analyzing the Fourier Transform Infrared (FTIR) spectra for samples S4-(*Cladophora* sp. control culture) and S4-T (treated culture of *Cladophora* sp.), it is clear that they share a very similar chemical backbone, though there are distinct differences in the intensity and presence of specific functional groups. This typically corresponds to hydroxyl (-OH) or amine (-NH) stretching. The increased intensity suggests a higher concentration of hydrogen-bonded groups or moisture in this sample S4-T. The peak in this region is significantly shallower and narrower, indicating a reduction in -OH or -NH content compared to S4-C. The range between 3000 - 2800 cm^{-1} wave number S4-C displays multiple small, sharp peaks. S4-T features a prominent, sharp downward spike near 2850 – 2950 cm^{-1} wave number. This is characteristic of C-H stretching (alkanes). The increased intensity in S4-T suggests a more pronounced hydrocarbon structure or a change in the polymer/molecule's side chains. In wave number 1750 – 1500 cm^{-1} Both samples show similar activity around often C=O or C=C stretching in between wave number range 1300 cm^{-1} – 700 cm^{-1} . These usually correspond to C-O stretching or P=O bonds depending on the material. Intensity Shift while the positions are identical, the relative transmittance in S4-T is slightly lower (meaning stronger absorption) for the peak near more or less 1100 cm^{-1} than in S4-C. The primary difference is in the hydration or hydroxyl content (higher in S4-C) and the aliphatic C-H content (more pronounced in S4-T). This often suggests that S4-T might be a "treated" or "modified" version of S4-C where some polar groups were removed or hydrocarbon chains became more visible.

Conclusion

From the above experiment it can be concluded that the audible sound frequency shows positive impact on algal *in vitro* culture. Audible sound application can act as a newly recognized ecofriendly stimulator that increases the growth

of algae as well as its metabolites. This harmless, low cost and ecofriendly method can be applied in large amount industrial algal production. By the FTIR chromatography it was found the essential biomolecules also increased in sound treated algae. So, the audible sound can play major role in both quantitative and qualitative improvement of the selected algal biomass.

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Conflict of interest

Authors do not have any conflict of interest.

References

1. Cardozo KHM, Guaratini T, Barros MP, Falcão VR, Tonon AP, Lopes NP, Campos S, Torres MA, Souza AO, Colepicolo P, Pinto E. Metabolites from algae with economical impact. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*,2007:146 (1–2):60–78.
2. Christwardana M, Hadiyanto H. The effects of audible sound for enhancing the growth rate of microalgae *Haematococcus pluvialis* in vegetative stage. *HAYATI Journal of Biosciences*,2017:24(3):149–155.
3. Fishman DB. 6 commercial products you probably didn't know are made with algae: You probably ate for breakfast. U.S. Department of Energy, 2017.
4. Ganguli K, Sil SK & Gupta S. Impact of audible sound on *in vitro* growth of Algae. *Ecology, Environment & Conservation*, 601-605.
5. Hait G, Bhattacharya K, Ghosh AK. A text book of botany (Vol. 1). New Central Book Agency (P) Ltd, 2012.
6. Heffner H, Heffner R. Hearing ranges of laboratory animals. *Journal of the American Association for Laboratory Animal Science*, 2007: 46(1):20–22.
7. Lee RE. *Phycology* (4th ed.). Cambridge University Press, 2008.
8. Manjunatha SS, Girisha ST. Characterisation of microbial biomass through Fourier Transform Infrared (FT-IR) spectroscopy. *International Journal of Botany Studies*, 2021:6(1):57–60.
9. Round FE. Dispersal continuity and phytogeography. In *The ecology of algae*, CUP Archive,1981:357–361.
10. Sathasivam R, Radhakrishnan R, Hashem A, Abd Allah EF. Microalgae metabolites: A rich source for food and medicine. *Saudi Journal of Biological Sciences*,2019:26(4):709–722.
11. Yamane H, Konno K, Sabelis M, Takabayashi J, Sassa T, Oikawa H. Chemical defence and toxins of plants. In L. Mander & H.-W. Liu (Eds.), *Comprehensive natural products II: Chemistry and biology*. Elsevier.doi.org, 2010:1:339–385.