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Short communication

Electrical frequency discrimination by fungi *Pleurotus ostreatus*

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ABSTRACT

We stimulate mycelian networks of oyster fungi *Pleurotus ostreatus* with low frequency sinusoidal electrical signals. We demonstrate that the fungal networks can discriminate between frequencies in a fuzzy or threshold based manner. Details about the mixing of frequencies by the mycelium networks are provided. The results advance the novel field of fungal electronics and pave ground for the design of living, fully recyclable, electronic devices.

1. Introduction

Fungal electronics aims to design bio-electronic devices with living networks of fungal mycelium (Adamatzky et al., 2021a) and proposes novel and original designs of information and signal processing systems. The living fungal electronic devices offer fault-tolerance and self-repairability featured in living systems, non-linear electrical properties (memfractance, capacitance, photoreactance, electrical oscillations) necessary for implementing analog electronic, neuromorphic and even digital (spike based) circuits, and the fungal circuits are capable of electrical responding to mechanical, optical, chemical and electrical stimulation. Mycelium bound composites (grain or hemp substrates colonised by fungi) are environmentally sustainable growing bio-materials (Karana et al., 2018; Jones et al., 2020b; Cerimi et al., 2019). They have been already used in insulation panels (Pelletier et al., 2013; Elsacker et al., 2020; Dias et al., 2021; Wang et al., 2016; Cárdenas-R, 2020), packaging materials (Holt et al., 2012; Mojumdar et al., 2021), building materials and architectures (Adamatzky et al., 2019) and wearables (Adamatzky et al., 2021b; Silverman et al., 2020; Karana et al., 2018; Appels, 2020; Jones et al., 2020a). To make the fungal materials functional we need to embed flexible electronic devices into the materials. Hyphae of fungal mycelium spanning the mycelium bound composites can play a role of unconventional electronic devices. Interestingly, their topology is very similar to conducting polymer dendrites (Cucchi et al., 2021; Janzakova et al., 2021). These properties originate not only from common topology (Pismen, 2020) but also from complex charge carrier transport phenomena. Therefore, it is not surprising that electrical properties of mycelial hyphae and conducting polymer filaments have similar electrical properties: proton hopping and ionic transport in hyphae *vs* ionic and electronic transport in polymers. Such transport duality must result in highly nonlinear voltage/current characteristics, which in turn, upon AC stimulation must result in generation of complex Fourier patterns in resulting current, as well as other phenomena relevant from the point of view of unconventional computing, e.g. stochastic resonance (Kasai et al., 2017).

We have already demonstrated that we achieved in implementing memristors (Beasley et al., 2022), oscillators (Adamatzky and Gandia, 2021), photo-sensors (Beasley et al., 2020), pressure sensors (Adamatzky and Gandia, 2022), chemical sensors (Dehshibi et al., 2021) and Boolean logical circuits (Roberts and Adamatzky, 2021) with living mycelium networks. Due to nonlinear electric response of fungal tissues, they are ideally suited for transformation of low-frequency AC signals. This paper is devoted to frequency discriminators and transformers, which are a significant contribution to the field of fungal electronics.

Electrical communication in mycelium networks is an almost unexplored topic. Fungi exhibit oscillations of extracellular electrical potential, which can be recorded via differential electrodes inserted into a substrate colonised by mycelium or directly into sporocarps (Slayman et al., 1976; Olsson and Hansson, 1995; Adamatzky, 2018). We used iridium-coated stainless steel sub-dermal needle electrodes (Spes Medica S.r.l., Italy), with twisted cables. In experiments with recording of electrical potential of oyster fungi *Pleurotus djamor* we discovered two types of spiking activity: high-frequency 6 mHz and low-freq 1 mHz (Adamatzky, 2018) ones. While studying other species of fungi, *Ganoderma resinaceum*, we found that the most common signature

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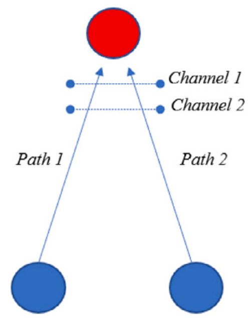

Fig. 1. Diagram showing connecting points to the fungi sample. The blue circles represent two places for the input signal injection whereas the red circle represents the ground.

of an electrical potential spike is 2–3 mHz (Adamatzky and Gandia, 2021). In both species of fungi we observed bursts of spikes within trains of impulses similar to that observed in animal central nervous system (Cocatre-Zilgien and Delcomyn, 1992; Legendy and Salcman, 1985). In Dehshibi and Adamatzky (2021) we demonstrated that information-theoretical complexity of fungal electrical activity exceeds the complexity of European languages. In Adamatzky (2022) we analysed the electrical activity of *Omphalotus nidiformis*, *Flammulina velutipes*, *Schizophyllum commune* and *Cordyceps militaris*. We assumed that the spikes of electrical activity could be used by fungi to communicate and process information in mycelium networks and demonstrated that distributions of fungal word lengths match that of human languages. Taking all the above into account it would be valuable to analyse the electrical reactions of fungi to strings of electrical oscillations, featuring frequencies matching those of the supposed fungal language. The present paper advances our research and development in (1) fungal electronics and (2) communication in mycelium networks by proposing novel and original designs of frequency discriminators based on living fungi.

2. Methods

A slab of substrate, 200 g, grains and hemp colonised by *Pleurotus ostreatus* (Ann Miller's Speciality Mushrooms, UK, https://www.annforfungi.co.uk/shop/oyster-grain-spawn/) was placed at the bottom of a 5 l plastic container. Measurements were performed in a classic two electrode setup. Electric contacts to the fungi sample were made using iridium-coated stainless steel sub-dermal needle electrodes (purchased by Spes Medica S.r.l., Italy), with twisted cables. Signal was applied with 4050B Series Dual Channel Function/Arbitrary Waveform Generators (B&K Precision Corporation) with a 16-bit vertical resolution. Signals featuring a series of frequencies – 1–10 mHz with a 1 mHz step and 10–100 mHz with a 10 mHz step – have been applied between two points of the fungi and measured with two differential channels on ADC-24 (purchased by Pico Technology, UK) high-resolution data logger with a 24-bit analog-to-digital converter. We have chosen these particular intervals of frequencies because they well cover frequencies of action-potential spiking behaviour of a range of fungi species (Adamatzky, 2018; Adamatzky and Gandia, 2021; Adamatzky, 2022).

For these frequencies, the sinusoidal signal was applied along two paths separately. Finally, mixing of signals was performed for 1 mHz base frequency applied on Path 1 and a series of frequencies on the Path 2. Frequencies used on Path 2 are 2, 5 and 7 mHz. Fast Fourier transform (FFT) was calculated with Origin Pro software. Blackman window function was used as it is best suitable for the representation of amplitudes (Dactron, 2003). Fuzzy sets for inference of new input data were constructed using "fuzzylogic 1.2.0" Python package.

3. Results

A response of the fungi sample to electrical stimulation is shown in Fig. 1a. In all measurements, electrical activity with frequency 50–200 mHz was observed even when substrates were not stimulated. This activity is attributed to endogenous oscillations of electrical potential of fungi (Adamatzky, 2018; Adamatzky and Gandia, 2021; Adamatzky, 2022).

Exemplary generations of higher harmonics are shown in Fig. 2b. In some cases presented on Fig. 3, 2nd harmonic is more damped than the 3rd harmonic. Generally, for frequencies below 10 mHz, higher amplitudes were observed for 3rd harmonic versus the 2nd.

The ratio of the 2nd to 3rd harmonic amplitudes was calculated to better illustrate the changes between them (Fig. 4a). The calculated ratios were then normalised to the ratio of harmonics at 10 mHz. Points at 30 and 50 mHz in 1 path, and 2 channel were treated as outliers because the ratios at these frequencies were disproportionally larger than those at other frequencies, which disturbed data visualisation. Besides, the omitted data points in the presented graph still support the observation that in general, below 10 mHz, the ratio of the 2nd and 3rd harmonics are smaller than for higher frequencies.

In the next step, Total Harmonic Distortion (THD) of the measured signal was calculated (Fig. 4b). THD is the ratio between the fundamental frequency amplitude V_0 and the amplitude of higher harmonics V_n:

$$\mathrm{THD}_F = \frac{\sqrt{V_2^2 + V_3^2 + V_4^2 + \cdots}}{V_1} \tag{1}$$

where V_n is the nth amplitude of the frequency of successive higher harmonic peaks observed in the Fourier spectra. Furthermore, normalisation to 100% of the THD parameter can be applied as follows:

$$\mathrm{THD}_R = \frac{\mathrm{TH}D_F}{\sqrt{1 + \mathrm{THD}_F^2}}, \tag{2}$$

where R in THD_R stands for "root mean square".

For the frequencies below 10 mHz, higher values of THD (up to 45.9%) can be observed in relation to higher frequencies, which tend to exhibit lower THD values (below 10%). The THD of a pure signal ranges between different values, for example a square wave features a THD of 48.3% and a triangular wave features a THD of 12.1%. This result may suggest changes in the dominant conductivity type: slower signals are more distorted and faster signals are much less distorted. Lower THD values are obtained, when the generation of higher harmonics of the modulated signal is low, hence the fungi sample has lower effect on its transformation. This effect is a consequence of a dual electric charge transport mechanism in mycelium. Furthermore, the changes occurring at low frequencies indicate, that slow physical phenomena (as diffusion) are critically responsible for the distortion of electric signals. This effect is similar to those observed in the case of solid-state memristor, however in the latter case the dependence is opposite (Przyczyna et al., 2022). It can be concluded that in the studied case at high frequencies only one, faster conductivity mode plays a significant role. Therefore, the nonlinear character of electric transport is much less pronounced and signal can apparently "fly through" the sample and can be transmitted across a macroscopic distance with low distortion.

As the changes of THD parameter below 10 mHz occurs in a rather continuous manner, arbitrary linguistic (very low, low, medium, high, very high, etc.) could be defined for ranges of obtained values. To cope with uncertainty of the classification, we can employ fuzzy sets theory (Zadeh, 1965). Following, membership function could be specified for the allocation of data into sets so that fuzzification of data could be implemented and allow for inference of given new input data into proper category (Mendel, 1995). Proposition for such sets is depicted in the background of Fig. 4b. Two sigmoidal sets were selected for the boundary and three Gaussian sets for the centre of the data.

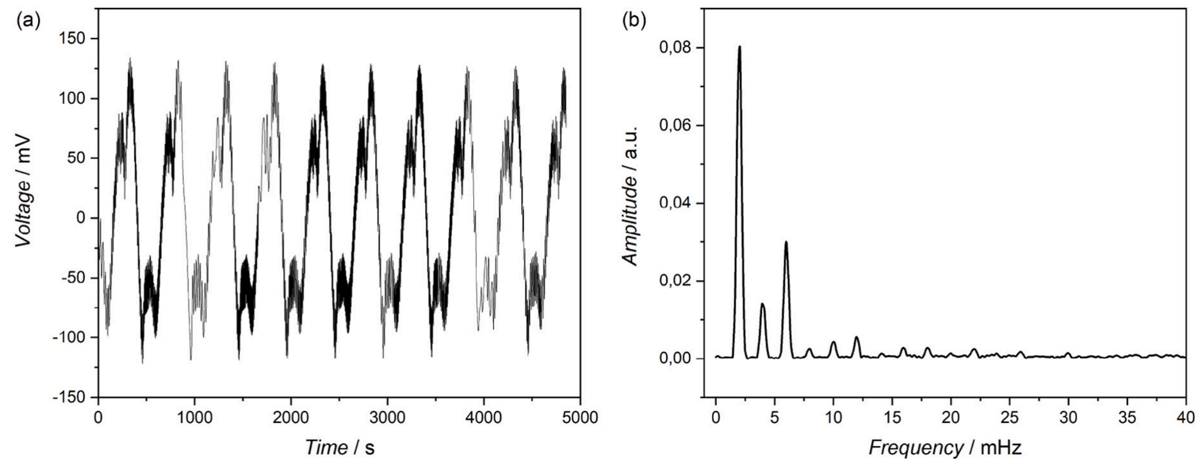

Fig. 2. Exemplary response of the fungal sample to 2 mHz, 10 Vpp sinusoidal electrical stimulation (a) and FFT for the same response.

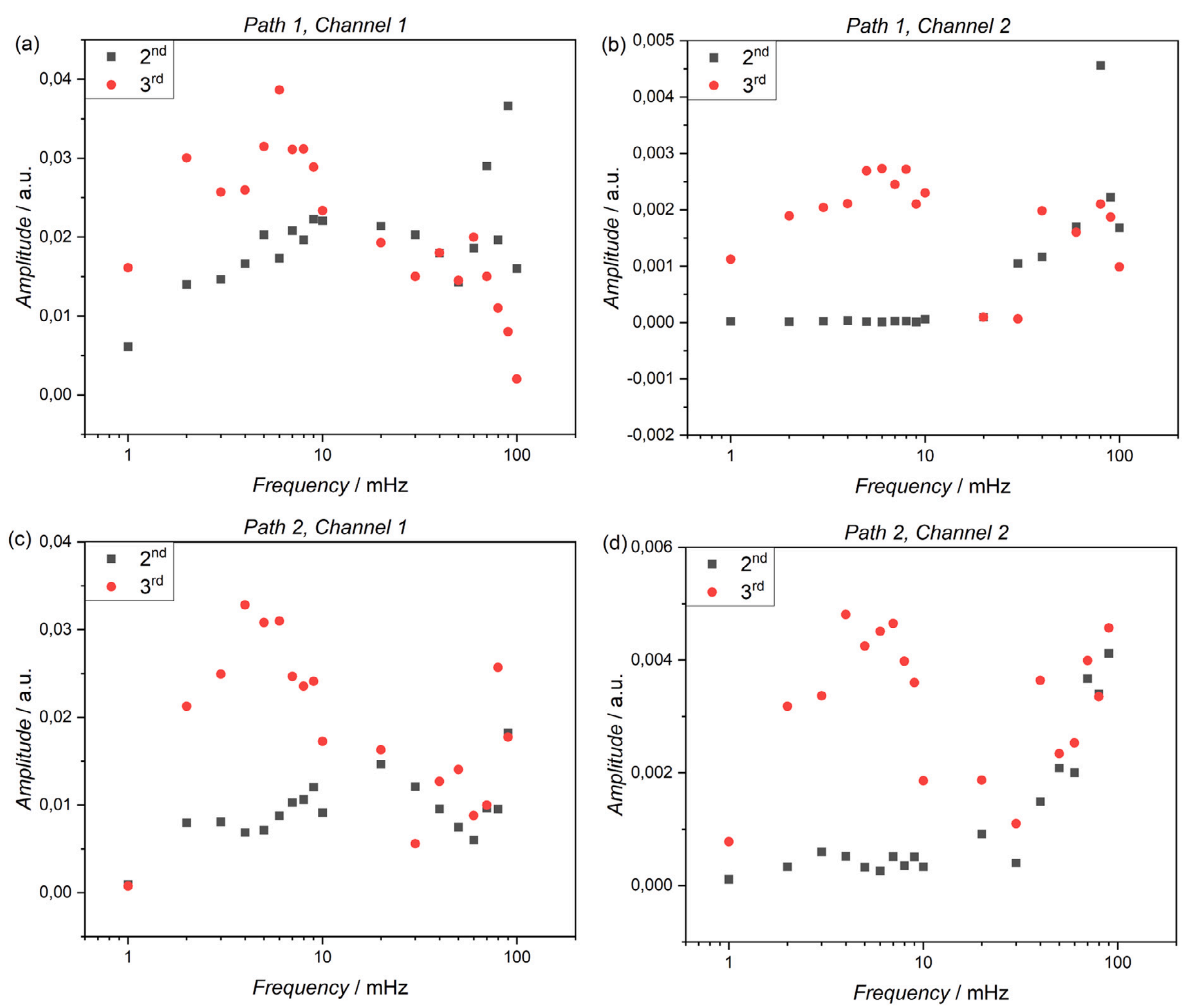

Fig. 3. Collection of 2nd and 3rd harmonic amplitudes obtained for the measured fungi response, for two signal paths and two differential channels.

The results demonstrate that, based on increase of the THD parameter or on the amplitude values of 2nd and 3rd harmonic components, signal discrimination based on its frequency could be realised.

After analysis of single signal paths, signals were applied to the two signal paths at once. Results show that with increasing frequency, further damping of the 2nd harmonic is achieved. Furthermore, satellite frequencies appear around base frequencies as well as around higher harmonics. For example, on Fig. 5b, for the mixing of 1 and 5 mHz signal, higher frequencies – 9 mHz and 11 mHz – around damped 10 mHz 2nd harmonic are present. This effect is present as well for the 1 mHz and 7 mHz mixed frequencies. The results indicate a nontrivial frequency mixing scheme, which may results in transport phenomena

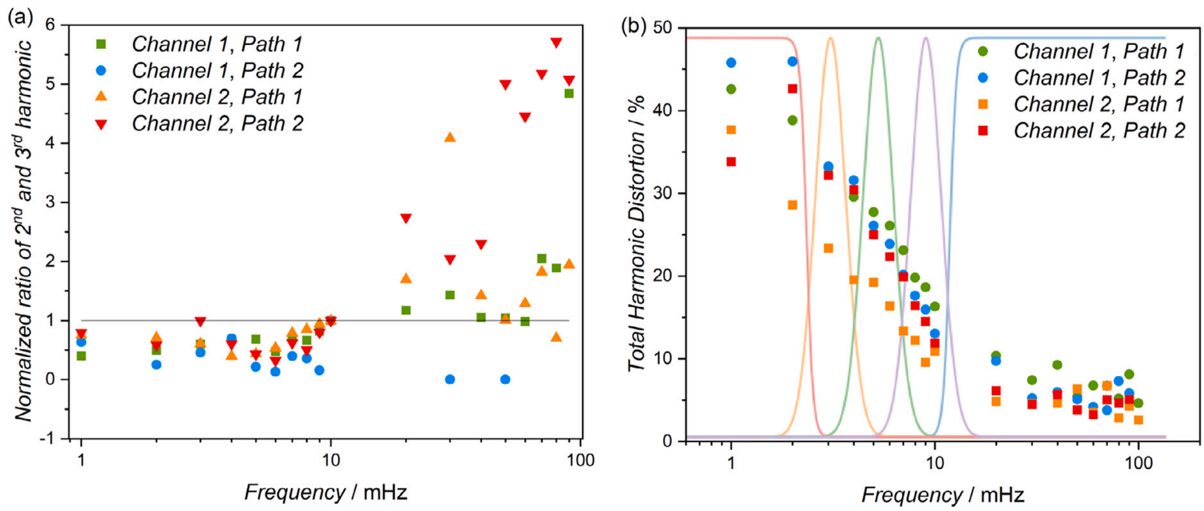

Fig. 4. Harmonic distributions. (a) Normalised ratios of 2nd vs. 3rd harmonics for analysed signals. Straight line marks threshold frequency. (b) Total Harmonic Distortion calculated for fungi sample. Proposition of the fuzzy sets is included in the background.

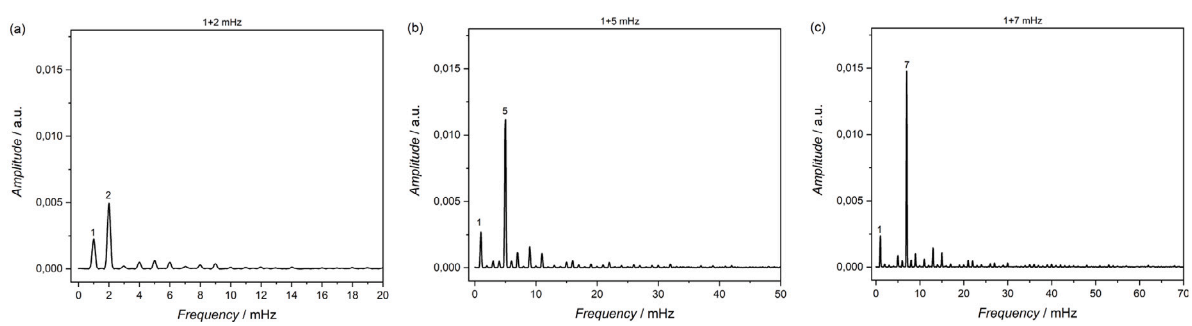

Fig. 5. Result of frequency mixing in the fungi samples. For each measurement, base 1 mHz driving signal was used on Path 1 (Fig. 1). For each successive measurement, higher frequency signal - 2, 5 and 7 mHz - was applied to the Path 2.

within highly branched network of mycelial hyphae. Such a transport can be a topic of future experiments (Zanin et al., 2018; Xiong et al., 2020).

4. Conclusion

We demonstrated that fungal mycelium networks modify frequencies of external electrical inputs. Damping of 2nd harmonic and amplification of the 3nd harmonic amplitudes below 10 mHz allow for frequency discrimination in a threshold manner. The frequency discrimination could occur in a continuous manner, with the help of the concepts of fuzzy logic based on THD parameter.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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