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Research Article

Piezoelectric Performance of Microbial Chitosan Thin Film Derived from *Aspergillus oryzae*

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Abstract. In this study, chitosan thin film derived from *Aspergillus oryzae* cell walls was fabricated and characterised. First, the chitosan from the fungal biomass was extracted (0.18 g/g) with 52.25% of degree of deacetylation obtained through Fourier transform infrared (FTIR) spectroscopy. Subsequently, several parameters of the chitosan thin film fabrication were optimised, including chitosan solution volume and drying temperature. Resultantly, the highest mechanical quality factor (3.22±0.012), the lowest dissipation factor (0.327±0.0003) and the best tensile strength (13.35±0.045 MPa) were obtained when pure chitosan was dissolved in 35 ml of 0.25 M formic acid and dried at 60 ˚C. In addition, the scanning electron microscopy (SEM) analysis presented a fine chitosan agglomerate distributed in the formic acid. The optimised fabricated, fungal-derived chitosan thin film was validated, recording a mechanical quality factor of 3.68 and dissipation factor of 0.248; both values were comparable to the synthetic polymer, polyvinylidene fluoride (PVDF) thin film. Thus, fungal-derived chitosan thin film can potentially be used as a piezoelectric material.

Keywords: *Aspergillus oryzae*; biopolymer; chitosan; deacetylation; fungi; piezoelectric; thin film

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1. Introduction

Chitosan is the most common renewable and biodegradable natural polymer after cellulose. This material is the building blocks of crustacean exoskeletons (crabs and shrimps) and fungal cell walls (*Aspergillus*, *Penicillium*, *Fusarium*, *Mucor*, *Rhizopus*, and *Choanephora*) that has been mass-produced for various purposes (Joseph *et al*. 2021; Hazmi *et al*. 2021). Studies have shown that the chitosan produced from crustacean wastes can range between 10% and 40%, while the fungal cell wall contains approximately 42% of chitosan (Bastiaens *et al*. 2019). Recently, fungal chitosan has been gaining popularity due to the ease of handling, low nutritional cost, and the feasibility of maintaining the desirable qualities. Recent research has explored the potential use of chitosan in biosensors (Akmal & Ahmad 2020). The non-centrosymmetric crystalline structure of chitosan makes it an excellent piezoelectric material to produce electrical charge from mechanical stress (Chorsi *et al*. 2019; Zamli *et al*. 2021). In addition, the chitosan structural ion orientation causes all dipoles to be parallel, resulting in maximum dipole moment (Rajala *et al*. 2018). Furthermore, chitosan is a sustainable piezoelectric biomaterial compared to the conventional lead zirconate titanate (PZT) and polyvinylidene fluoride (PVDF) due to superior properties such as biodegradability, biocompatibility, and low toxicity (Mishra *et al*. 2019). The synthesis of PVDF could release toxic fluoride gas that makes its life cycle not to be sustainable (Lohmann *et al*., 2020)

Furthermore, not all fungi contain naturally synthesized chitosan within their cell wall. Only a few fungi strains from *Zygomycetes* class contain both chitin and chitosan in their cell wall (Dhillon *et al*., 2013). 20-50% of fungi strains exhibit low concentrations of chitosan in its cell walls. *Aspergillus oryzae* from *Ascomycetes* class composed of chitin and cellulose (Dhillon *et al*., 2013). Therefore, it could be a good source of chitosan via the

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deacetylation process. Chitosan extraction and properties enhancement are achieved through chitin deacetylation using alkali and acidic treatment. Furthermore, chitosan can be purified from the fungal cell wall since this compound is soluble in acidic solutions (e.g., acetic acid, formic and lactic) (Zamani *et al*. 2010). However, acetic acid is not the best extraction solvent for the complete chitosan extraction from the fungal cell wall. Alternatively, dilute sulphuric acid has been proposed as a solvent for chitosan extraction from the cell wall of zygomycetes (Zamani *et al.* 2010). The principle of this method is based on the temperature-dependent solubility unique to only chitosan and no other cell components.

To achieve the best electrical and mechanical properties, it is critical to use the right processing conditions to fabricate thin films. To date, there are no studies on the optimising chitosan thin film from fungal biomass for piezoelectric application. Among important parameters to consider for optimising thin film fabrication are (1) drop-casting volume of chitosan solution, (2) temperature of oven drying and (3) concentration of acid solvent. Drop-casting volume of chitosan solution is significant as it influences the thickness of thin film. The thickness and drying temperature are two crucial parameters that could enhance the crystallinity and consequently would improve the piezoelectric properties of thin film (Chen *et al.* 2019).

In the present study, the fabrication of microbial chitosan thin film for piezoelectric application was investigated. This work also involves the use of bioreactor, which is important for scaling up fungal biomass production. The fabrication of chitosan thin film was further optimised for finding optimum electrical (mechanical quality factor, dissipation factor) and mechanical properties (tensile strength) of film. The findings of this study may provide a sustainable source of chitosan due to the seasonality of crustacean-based chitosan. In addition, the fabricated chitosan thin film is a promising alternative biomaterial for portable piezoelectric-based energy harvesting or nanogenerator and sensors.

2. Materials and Methods

2.1. Cultivation of fungi

The fungal strain *Aspergillus oryzae* was cultured on potato dextrose agar (PDA) in Petri dishes Fig.1(a) and incubated at 25 ˚C for five days and stored at 4 ˚C until further inoculation into medium. Media consisted of 0.03 g/ml glucose, 0.003 g/ml peptones and 300 ml distilled water was prepared and sterilized as pre-inoculum culture medium and cultivation medium (Abdel-Gawad et al. 2017). The pre-inoculum culture of *Aspergillus oryzae* was incubated in 500 ml Erlenmeyer flask with 150 rpm at room temperature for 3 days. The pre-inoculum culture medium was then transferred into a 2000 ml (Fig.1(b)) bioreactor under aseptic condition with the aid of Bunsen burner. The impeller speed of the reactor was set at 200 rpm and the cultivation medium was maintained at pH 5.5. The temperature of the reactor was set at 26˚C with aeration rate of filtered air intake at 500 ml/min. The bioreactor cultivation was performed for 3 days. Sampling was done for every 12 h. After 3 days of cultivation, the fungal biomass was harvested via filtration and washing. The fungal mycelium was dried at 60˚C overnight in the oven and was further used for chitosan extraction.

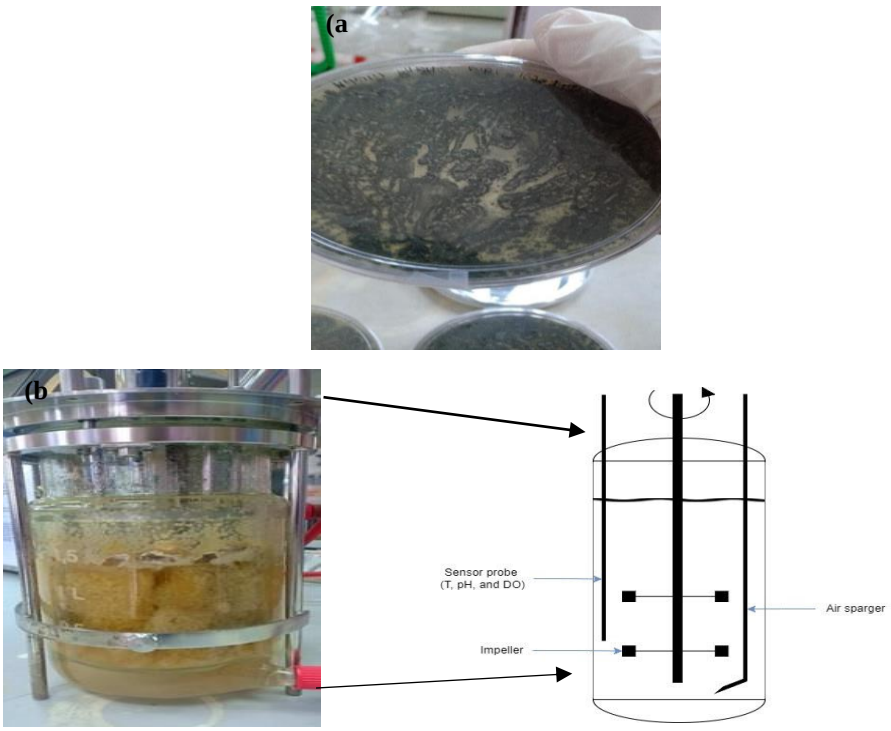

Fig 1. *Aspergillus oryzae* culture on PDA (a) that was used to inoculate the pre-inoculation culture media (b) submerged cultivation of *Aspergillus oryzae* in 2000 ml stirred tank bioreactor.

2.2. Chitosan extraction from fungal biomass

Chitosan was then extracted from the harvested fungal biomass using sodium hydroxide (NaOH) and acidic acetic acid (CH_3COOH) treatments as proposed by Abdel-Gawad *et al*. (2017). Chitosan yield was calculated based on the following equation (Equation 1):

$$Chitosan\ yield\ (\%, w/w) = \left[\frac{dry\ weight\ of\ obtained\ chitosan}{dry\ weight\ of\ sample}\right] \times 100\% \quad (1)$$

2.3. Optimisation of thin film fabrication

The production of chitosan thin film was optimised via one-factor-at-time (OFAT) method with two parameters which are (1) drop-casting volume of chitosan solution and (2) temperature of oven drying and three responses which are (1) mechanical quality factor, Q_m, (2) dissipation factor, *tan δ* and (3) tensile strength. Chitosan solution was prepared by dissolving pure chitosan powder (1 g) in 99 ml formic acid solution (1% *v/v*). Then, the chitosan solution was casted onto Petri dishes at different volumes (20, 25 and 30 ml) while the drying temperature (60 ˚C) and acid solvent concentration (0.25 M) remained constant. After the best drop-casting chitosan solution volume was determined, the drying temperature was varied (50˚C, 60˚C and 70˚C). The thin film peeled off naturally after three to four hours.

The volume and temperature ranges were chosen based on the preliminary studies by Praveen et al., (2017) and Suderman *et al*., (2016). The threshold for volume of chitosan solution was at 25 ml as below this value, the volume of chitosan solution could not completely cover the surface of petri dish.

2.4 Glucose consumption

High performance liquid chromatography (HPLC) (Waters) was used to analyse the sampling (spent medium) collected from the bioreactor to determine the glucose consumption. ZORBAX Eclipse Plus C18 column (Agilent) was used with ultrapure water as the liquid mobile phase to carry the analyte. The spent media was prepared by diluting it into distilled water with dilution factor of 10 and filtered using syringe filter. Ultra-pure water (UPW) was used as the mobile phase, which was eluted in less than 10 minutes. The column temperature was set to 35 °C and the flow rate was 0.3 ml/min (Sahebi *et al.* 2019).

2.5 Degree of deacetylation (DDA)

The chitosan deacetylation degree was determined via Fourier transform infrared (FTIR) spectroscopy (4000 cm^{-1} – 400 cm^{-1}). The functional groups, amide-I band (1655 cm^{-1}) and hydroxyl (OH) group (3450 cm^{-1}) in chitosan, were used as internal references in this analysis. The DDA was calculated using Equation 2, where A_{1655} and A_{3540} are FTIR absorbance values at 1655 and 3540 cm^{-1}, respectively (Lin et al., 2019):

$$DDA\% = 100 - \left(\frac{(A_{1655} - A_{3450}) \times 100}{1.33}\right) \quad (2)$$

2.6 Scanning Electron Microscopy (SEM)

Fungal-derived chitosan thin film surface morphology was analysed and photographed using scanning electron microscopy (SEM) (ICON ANALYTICAL, FEI with Model QUANTA 200 instruments operating at 8kV) at a magnification of 10000x.

2.7 Tensile strength

The Universal Tensile Machine (UTM) linked to Trapezium X Software was set to run the chitosan thin film sample at 20 mm/min to determine the tensile strength. The chitosan thin film is cut into a dimension of 20 mm x 35 mm. All the analyses were performed in triplicate.

2.8 Mechanical quality factor (Q_m) and dissipation factor (tan δ)

The chitosan thin film sample (20 mm x 20 mm) was connected to the handheld LCR (inductance, capacitance and resistance) meter (Keysight U1730C Series) using a copper wire as a conductor and charged with a constant frequency of 10 kHz. The mechanical quality factor (Q_m) and dissipation factor (*tan δ*) at a frequency was measured in short time intervals (~1-2 min) until a constant value was obtained. All the analyses were performed in triplicate.

3. Results and discussion

3.1 Aspergillus oryzae cultivation in the bioreactor and chitosan extraction from the fungal biomass.

The spent media from sampling were analysed using HPLC to determine the consumption of glucose in the media by fungi during the cultivation. Based on the cell dry weight calculation, the fungal biomass concentration obtained was 0.005 g/ml. Meanwhile, from the sugar consumption analysis, the concentration of glucose in the spent medium at the end of fungal cultivation was determined to be 0.0024 g/ml. Therefore, the bioreactor cultivation of fungi demonstrated high consumption glucose by 92%. This could be attributed to the improved efficiency of fungal metabolism and growth in bioreactor. Relative to shake flask cultivation, the cultivation in bioreactor system involved controlled key parameters for microbial growth, which includes of aeration, agitation, dissolved oxygen content and pH. The details of the findings are tabulated in Table 1.

3.2 Degree of deacetylation (DDA) of fungal chitosan

The absorbance of chitosan obtained is depicted in Fig 2. The presence of two separate peaks at (1587.08 cm^{-1}) and OH group (3366.06 cm^{-1}) in chitosan are used as internal references. It is important to note that the electrostatic interaction that occurs when hydroxyl and amide groups are present causes these groups to have piezoelectric capabilities (Karan *et al*. 2018). When an external force is applied, the crystalline structure of chitosan forms a dipole moment, which caused this phenomenon to occur (Karan et al. 2018).

Table 1
Chitosan extraction from *Aspergillus oryzae* biomass harvested from bioreactor cultivation.

Parameter	Value
Concentration of fungal biomass (g/ml)	0.005
Glucose consumption (g/ml)	0.0276
Yield of fungal biomass (g/g)	0.181
Concentration of extracted chitosan (g/ml)	0.0005
Chitosan yield (%)	10

The efficient extraction of chitosan from fungal biomass depends on the degree of deacetylation (DDA) (Ghormade *et al*. 2017). Therefore, the FTIR absorbance spectra results were used to calculate the DDA of the chitosan. The DDA percentage obtained via FTIR spectroscopy must be at least 50% to confirm that the extracted product is chitosan. In this study, the absorbance obtained were 0.060 and 0.095 respectively and used to calculate the DDA using Equation 2. The DDA value of chitosan from *Aspergillus oryzae* was 52.5%, which demonstrated that chitosan was successfully extracted from fungal biomass and comparable to the findings of other researchers at 55.23% (Jebur *et al.* 2019). Remarkably, the removal of the acetyl group by the DDA is essential for assuring that the chitosan is highly soluble in the suitable solvent for subsequent thin film production (Yusharani *et al.* 2020).

Principally, a high DDA can affect the properties of chitosan by increasing its crystallinity. Furthermore, a material with a high DDA will have a greater elastic modulus and tensile strength when fabricated into a thin film (Yuan *et al.* 2011). Moreover, Abdel Gawad et al. reported that longer incubation time and lower temperature could increase DDA value (Abdel-Gawad *et al*. 2017), which was applied in the current study to obtain favourable outcomes.

3.3 Drop-casting volume of chitosan solution

It was critical to figuring out whether chitosan, while operating as a piezoelectric material and being subject to repetitive mechanical actions and vibrations, could withstand sustained strains or overloads. Hence, tensile strength test was conducted to determine the tensile strength of chitosan drop-casted with varies chitosan volume (20 ml, 25 ml, 30 ml). The maximum tensile strength of 18.33±0.3 MPa (see Fig. 3) was recorded when 30 ml of chitosan solution was cast onto the Petri dish. However, there was a gradual decrease in tensile strength corresponding to the increased chitosan volume (35 ml) and film thickness. Theoretically, the polymer molecular chains are restricted within its layer and aligned with increasing thickness, lowering the stretchability of the film. At a particular thickness, the film's mechanical characteristics will therefore decline (Wang *et al*. 2020).

The mechanical quality factor, $Q_{m,}$ is the main extrinsic factor that improves the piezoelectric response of the thin film. Excellent piezoelectric material is expected to possess superior mechanical quality factor within the crystal unit in the thin film, owing to the material ability to increase dipole moments orientation. The mechanical quality factors of chitosan thin film cast with different solution volumes are demonstrated in Fig. 3. The highest mechanical quality factor, $Q_{m,}$ obtained was 3.22±0.012. The increasing trend in Q_m value was proportional to the thickness of chitosan thin film, indicating the improvement in material densification and crystallinity. Consequently, dipole-dipole movement is facilitated, thus, enhancing piezoelectric response (Singh *et al*. 2015).

The average value of the dissipation factor (*tan δ*) for thin films at three different drying temperatures is also shown in Fig. 3. The loss-rate energy of piezoelectric material depends on the dissipation factor (tan *δ*). The lowest dissipation factor, 0.327±0.0003, was recorded by the thin film prepared with 35 ml of chitosan solution. The reduction in dissipation factor with increased chitosan volume is likely due to the increment of localised charge carriers that reduce the dissipation factor within the chitosan crystalline structure (Rahman *et al*. 2021).

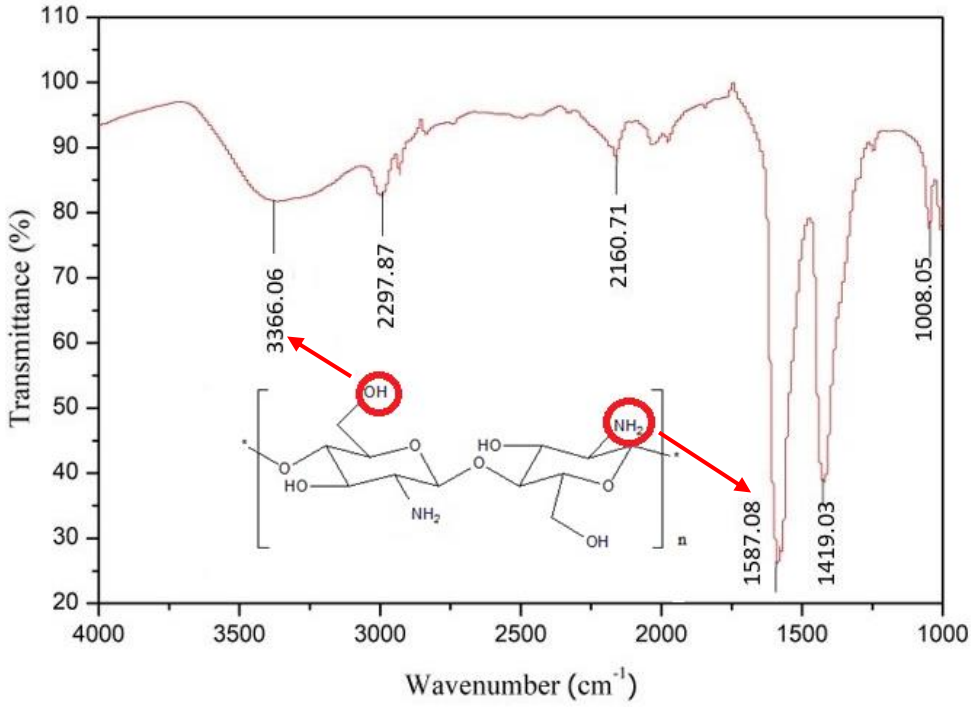

Fig 2. FTIR spectrum in fungal chitosan obtained from this project

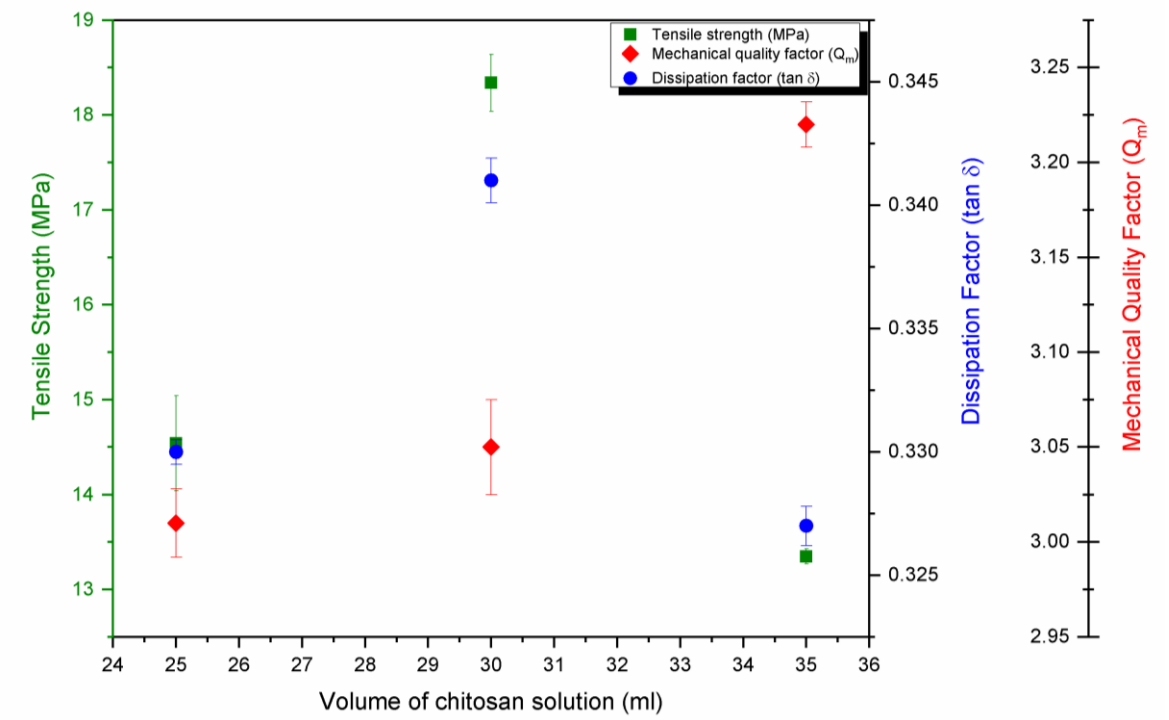

Fig 3. Tensile strength, mechanical quality factor (Q_m) and dissipation factor (tan δ) with different drop-casting volume of chitosan solution

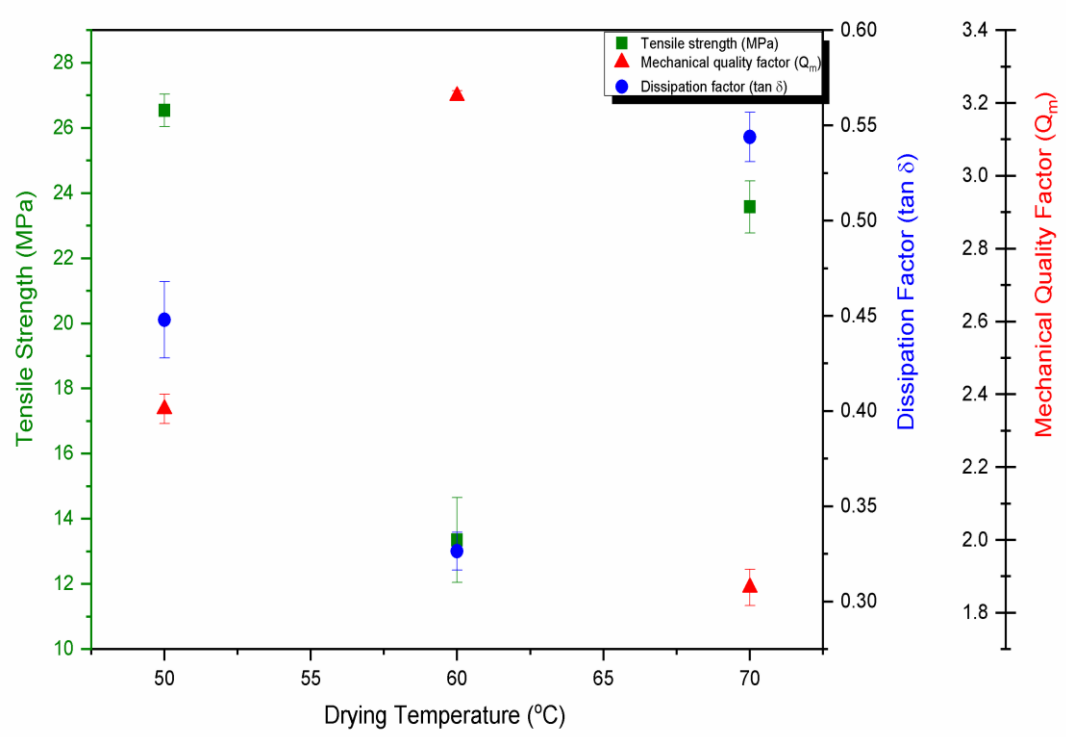

Fig 4. Tensile strength, mechanical quality factor (Q_m) and dissipation factor (tan δ) chitosan thin film dried with various oven drying temperature.

3.4 Drying temperature

The optimal drying temperature for the thin film was determined by testing different oven temperatures: 50 ˚C, 60 ˚C, and 70 ˚C. A fluctuation in tensile strength was identified at different temperatures where the thin film dried at 50 ˚C recorded the maximum tensile strength (26.54±0.4 MPa) compared to other temperatures (Fig. 4).The tensile strength of the films was increased as a result of the dense polymer chain packing at low drying temperatures (Akmal *et al.* 2018). For piezoelectric applications, it should be emphasised that the thin film tensile strength shouldn't be excessively strong or weak (Li et al. 2021). In order to increase the tensile strength of chitosan thin film as a piezoelectric material, it is crucial to control the thickness and drying temperature.

The value of the mechanical quality factor, Q_m, for thin films at three different drying temperatures was recorded in Fig. 4. There was an increasing trend in mechanical quality from 50 ˚C to 60 ˚C, but the value began to decrease at 70 ˚C. The best result was obtained at 60 ˚C with a value of 3.22±0.014. This finding could be due to the uneven heat distribution during the drying process and the lack of drying efficiency of the oven. An earlier study has reported that the mechanical quality factor for a piezoelectric thin film critically relies on the quality and orientation of the thin film, which in turn depends on the solvent deposition method and processing conditions (Hasan *et al*. 2021).

Fig 4. also demonstrates the average value of the dissipation factor (*tan δ*) for thin films at three different drying temperatures. The lowest dissipation factor was exhibited at 60 ˚C with a value of 0.327±0.0008. Joshi et al. hypothesised that the dissipation loss of piezoelectric materials occurs at different processing temperatures (Joshi, Hung & Vengallatore 2014). A higher temperature can increase the dissipation factor, potentially giving rise to lattice vibration and some phonons that interact with the charge carriers, resulting in electron-phonon scattering.

3.5. Chitosan thin film with selected optimised parameter

The optimal parameters were determined based on the highest Q_m and lowest *tan δ* value obtained in this study to validate the fungal chitosan. These responses were considered important indicators in characterising the chitosan as a good piezoelectric material (Damjanovic 2018). Therefore, the selected optimised parameter for this project was the thin film with 35 ml solvent volume and 60 ˚C drying temperature.

The chitosan thin film was characterised by assessing the physical and electrical properties, including surface morphology, mechanical quality factor (Q_m) and dissipation factor (tan δ). In addition, the mechanical quality factor and dissipation factor of fungal chitosan were compared with commercial pure chitosan and PVDF.

3.5.1 Surface morphology of fabricated chitosan thin film

The SEM was conducted to observe the surface morphology of the chitosan thin film (Fig. 5(a)). The dissolution of chitosan in the formic acid was observed from the SEM images taken from the top view operating at an acceleration voltage of 8 kV. It can be observed that the pure chitosan structure is less dense with non-uniform distribution of aggregate. The structure of fungal chitosan (Fig. 5b), on the other hand, was denser and more compact, which is consistent with the past research (Cai *et al*. 2019). As previously discovered, fungal chitosan does not exhibit the microfibrillar structure, distinguishing itself from chitin (Paiva *et al*. 2021). Therefore, it is confirmed in this study that chitosan was successfully extracted from *Aspergillus oryzae* since fungal chitosan did not exhibit microfibrillar structure. In addition, a uniform surface morphology of fungal chitosan could improve dipole-dipole interactions, hence, hence boosting the thin film's piezoelectricity (Akmal & Warikh 2021)

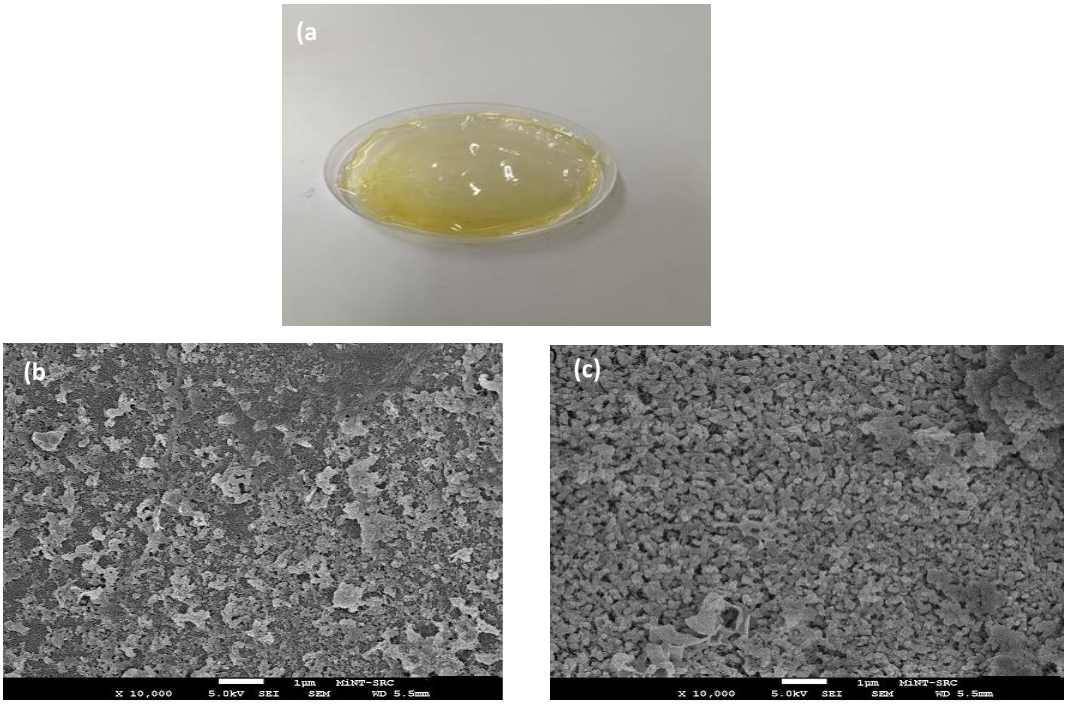

Fig 5. (a) Chitosan thin film derived from *Aspergillus oryzae*, (b)SEM image of commercial pure chitosan (c) SEM image of fungal chitosan at 10,000x magnification.

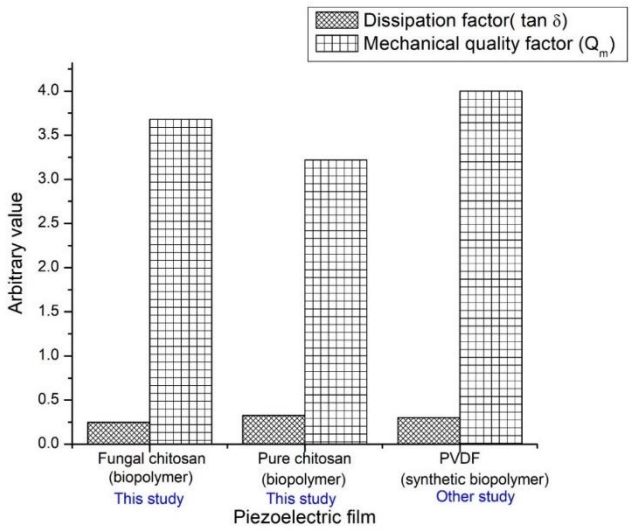

Fig 6. Mechanical quality factor value fungal chitosan compared film with pure chitosan from this study and benchmarked with polyvinylidene fluoride (PVDF) film, which has been widely used in piezoelectric application (Bhuvaneshwari et al. 2011 & Toda et al. 2001).

3.5.2 Mechanical quality factor and dissipation factor.

The Q_m values recorded for pure and fungal chitosan were 3.22 and 3.68, respectively as can be seen in Fig. 6. The mechanical quality factor of fungal chitosan was slightly higher than pure chitosan, indicating the former was more efficient in producing electrical force from the mechanical force than the latter. The difference in pure and fungal chitosan might be associated with different surface morphology, which may abruptly increase or reduce the Q_m values (Cai *et al*. 2019). Notably, PVDF film, a typical piezoelectric polymer, performed marginally better than fungal chitosan film (Toda *et al.* 2001); Bhuvaneshwari *et al.* 2011). The results of Q_m measurements, however, are acceptable for piezoelectric materials because they showed that chitosan could withstand repeated mechanical vibrations at resonance frequencies (Shekhani & Uchino 2015).

As shown in Fig. 6, the dissipation factor of pure chitosan and fungal chitosan were also compared with PVDF in order to confirm the dissipation factor (*tan δ*) of the fabricated chitosan thin film. In this study, the dissipation factors of the pure chitosan and the fungal chitosan were 0.327 and 0.248, respectively. Pure chitosan recorded a higher tan δ value than fungal chitosan and PVDF. This is most likely due to the decrement of dipole moment interaction in the crystalline structure. In addition, the dissipation factor of pure chitosan may increase due to lesser phonons interaction in the lattice structure due to factors like surface inhomogeneity which coincides well with SEM result. This phenomenon would lead to the decrease in localised charge carriers, which reduced the *tan δ* within the crystalline structure of the chitosan, thus reducing the piezoelectricity (Amarande *et al*. 2007). Conclusively, fungal chitosan film is a promising material for the piezoelectric application.

4. Conclusion

This study had successfully extracted 5 g/L of fungal biomass that yielded 0.18 g/g (w/w) chitosan, with a calculated DDA of 52.5%. Chitosan at a volume of 35 ml dissolved in formic acid exhibited the best tensile strength 13.35±0.045 with a mechanical quality factor of (3.22±0.012) and the lowest dissipation factor (0.327±0.0003). Meanwhile, the chitosan thin film oven-dried at 50 ˚C recorded the best tensile strength (26.54±0.4 MPa), while the highest mechanical quality factor (3.22±0.014) was obtained at 60 ˚C and the lowest dissipation factor (0.327±0.0008). Both parameters that were optimised in this study significantly impacted the mechanical and electrical properties of the chitosan thin film. Moreover, the distribution of fine aggregate on chitosan film surface morphology was evident in the SEM analysis. Apart from that, the fabricated fungal chitosan with optimised parameters possesses the tensile strength of 13.35 MPa, the mechanical quality factor of 3.68 and the dissipation factor of 0.248. These findings indicated that the performance of fabricated fungal chitosan is comparable to the commercial PVDF thin film. Therefore, it was proven that fungal chitosan thin film is a promising alternative biomaterial for piezoelectric application.

Acknowledgements

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