Characterization of chitosan from extracted fungal biomass for piezoelectric application

F. B. Ahmad1*, M. H. Mazlati Akmal2, A. Amran1 and M. H. Hasni1
1Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, Kuala Lumpur, Malaysia
2Department of Science in Engineering, Faculty of Engineering, International Islamic University Malaysia, Kuala Lumpur, Malaysia
*e-mail: farahahamad@iium.edu.my

Abstract
Conventional piezoelectric materials from piezoceramic and polymer are non-renewable and could be toxic in nature, which limit its application in biomedical application. Chitosan, which is a natural polysaccharide, has the potential to be used as piezoelectric biomaterial which may provide the solution for toxicity, non-biodegradability and non-biocompatibility issues of conventional piezoelectric materials. Chitosan may be produced sustainably through extraction from fungal cell walls. This study aims to characterize chitosan extracted from fungi Aspergillus oryzae for piezoelectric application. A. oryzae was cultivated on modified Sabouraud dextrose broth medium. Alkaline treatment was performed on fungal biomass using 1 M NaOH for extraction and deacetylation of chitosan at 100 °C for 1 hour. Fourier transform infrared spectroscopy results showed that the broad absorption band that corresponds to hydrogen bonded O-H stretching vibrations overlapped with N-H stretching band. X-ray diffraction analysis confirmed the semicrystalline nature of the chitosan sample. Piezoelectric properties can be attributed to intrinsic molecular polarization arising from the non-centrosymmetric crystal structure.

1. Introduction
Piezoelectric effect happens when the piezoelectric element is deformed, where electric polarization occurs in proportion to the pressure, in which a potential difference occurs until the space charge corrects this polarization [1]. Material that possess piezoelectric properties can be used in various application as smart material in energy harvesting, biomedical devices and actuator.

The conventional piezoelectric materials are piezoceramics (e.g., lead zirconate titanate (PZT)) and piezoelectric polymers (e.g., poly(vinylidene fluoride) (PVDF)). Piezoceramics can typically generate a very high piezoelectric coefficient (from 200 to 350 pC/N), however, it is non-renewable, not biodegradable and can pose cytotoxicity to human cells [2-4]. PVDF is flexible, non-toxic and can generate significant piezoelectricity [2]. However, PVDF is also non-biodegradable polymer, expensive and may also cause the release toxic gases such as hydrogen fluoride its syntheses and decomposition processes [3, 5].

Piezoelectric material could be derived from biomaterial that may provide the solution for toxicity, cost and non-biodegradability issues of the conventional piezoelectric materials such as chitosan. Chitosan is derived from chitin, in which both materials were reported to possess piezoelectricity. Chitosan is a polymer, which contains β-1-4 linked 2-amino-2-deoxy-D-glucopyranose repeat units, derived from the N-deacetylation of chitin [6]. Chitin is the natural abundant polysaccharide found in the structural components of the cuticles of crustaceans, insects and mollusks, as well as cell wall of mushroom and fungi [3, 7]. The piezoelectric properties of chitin are attributed to intrinsic molecular polarization arising from the non-centrosymmetric crystal structure of both α- and β-chitin polymorphs [3]. Chitin films
derived from prawn shell, crab shell and squid pen have been studied for piezoelectric application which includes flexible green electronics, nanogenerator and paper-type speaker and microphone [3, 7-9].

The use of chitosan as piezoelectric biomaterial is more advantageous than chitin as the solubility of chitin is limited. The solubility of chitosan will allow easy mixing biocomposite film preparation [5, 10]. Chitosan also possess much higher piezoelectric coefficient at 25 pC/N than chitin (0.2–1.5 pC/N) and comparable to PVDF [2, 10]. Biodegradability of the piezoelectric biomaterial is an added value of the use of chitosan as there will be no toxic wastes released throughout its lifecycle.

However, there are extremely limited studies on chitosan-based piezoelectric materials. The cell wall of zygomycetes such as fungi is considered to be mainly consists of chitin and chitosan (20–50%) [11]. Therefore, chitosan may be extracted from fungal cell wall as a sustainable and renewable source, in which its availability will not be limited by climate, fishing activities and seasonal demand like chitosan derived from crustaceans. In addition to that, chitosan extraction from fungi does not require demineralization and decolorization steps in the extraction of chitosan from crustaceans [12]. Microbial-derived chitosan could be easily scaled up in incubating tank for commercial production without requirement of large land area. The extraction of chitosan from fungi is achieved by combination of mild alkaline and acidic treatments, therefore, it is considered as a green processing [12]. Alkaline treatment is undertaken in order to remove soluble alkali material like glucan and protein that are present in fungal biomass [13]. The highest yield of chitosan extracted from *Aspergillus niger* via hot alkaline and acetic acid treatment was 0.91 g/L from Sabouraud Dextrose Broth media containing 8% (w/v) glucose with 12 days of cultivation [6].

This study aims to characterize chitosan extracted from fungi *Aspergillus oryzae* for piezoelectric application. To date, there are no studies exploring the potential of utilizing chitosan derived from *A. oryzae* for piezoelectric application.

### 2. Materials and methods

#### 2.1 Microorganisms

Fungi strain *Aspergillus oryzae* was obtained from the culture collection of INBIOSIS UKM, Malaysia. The fungal culture was maintained on potato dextrose agar (PDA) at 4 °C [14]. *A. oryzae* was cultivated on Sabouraud Dextrose Broth (8% glucose) media with the addition of 5% (w/v) of yeast extract as the nitrogen source in 250 mL Erlenmeyer flasks for 9 days at 25 °C with 150 rpm agitation [6].

#### 2.2 Fungal harvesting and chitosan extraction

At the end of the cultivation, the fungal biomass was harvested by filtration and washing. The fungal biomass will be dried in the oven at 60 °C. Fungal biomass was milled in homogenizer.

Extraction of chitosan from fungal biomass involves deproteinization and deacetylation by alkaline treatment (sodium hydroxide (NaOH)) and solubilization by dilute acetic acid. The alkaline treatment involved the use of NaOH (1:40 w/v) at 1 M with extraction temperature of 90 °C for 3 h [12]. The solid residue from alkaline treatment was called alkali-insoluble material (AIM).
AIM was separated by centrifugation (6000 rpm, 15 min), washed with distilled water and recentrifuged until reaching to a neutral pH. To isolate chitosan from the AIM, the residues was further extracted using 10% v/v acetic acid (1:40 w/v) at room temperature for 6 h on a rotary shaker (200 rpm), then the acid insoluble residue was discarded by vacuum filtration [12]. Chitosan is in the filtrate in which the pH of the filtrate will be adjusted to pH 9.0 with 4 M NaOH solution, and recentrifuged [12]. The precipitated chitosan was washed with distilled water, and dried at 60 ºC to a constant weight.

2.3 Fourier-transform infrared spectroscopy (FTIR)
FTIR spectrum of the chitosan was recorded in the 4000-400 cm⁻¹ region.

2.4 X-Ray Diffraction (XRD)
XRD analysis on chitosan sample was conducted using X-Ray Diffractometer Empyrean with Cu-Kα radiation at λ = 1.5406 Å in a scanning range of 2θ angles from 5° to 80°.

2.5 Field Emission Scanning Electron Microscopy (FESEM)
The extracted chitosan samples were examined and photographed in FESEM using ICON ANALATYCAL, FEI with Model QUANTA 200 instruments.

3. Results and discussion

3.1 Extraction of chitosan from fungal biomass
Chitosan investigated in this study was extracted from fungal cell wall of Aspergillus oryzae cultivated on glucose-based media. The cell walls of A. oryzae was reported to be composed of chitin and polysaccharide of glucose, galactose and mannose, in which the type of glucan identified was β-1,3- and α-1,4-linked polysaccharides [15]. The structural components (chitin, chitosan and β-glucan) and interstitial components (mannoproteins, galactoproteins, xylomannoproteins and glucuronoproteins) of fungal cell wall can be separated hot water or dilute alkali treatment, as the interstitial components and other cell components (proteinaceous) are soluble in alkaline solution [16]. Chitosan is separated from the remaining structural components (chitin and β-glucan) by acid extraction, due to its solubility in acidic solutions [16]. Therefore, extraction of chitosan from fungal biomass comprises of alkaline treatment for deproteinization and deacetylation and dilute acetic acid treatment for chitosan separation. Deproteinization step consists of dissociating the chitin-glucan complex and degrade proteins, where the alkali-insoluble material (AIM) is made of chitosan, chitin, and β-glucans [12]. Chitosan is separated from AIM via solubilization using acidic solution such as acetic acid, which promotes protonation of the amino groups of the chitosan chains [12]. The yield of chitosan from A. oryzae biomass in this study was 15.12% (w/w). The extraction of chitosan from A. niger biomass by Abdel-Gawad et al. showed that the optimum yield from two-steps alkaline and acid treatment was 8.12% [12], in which the result of this study was higher.

3.2 Chemical properties of fungal chitosan
FTIR analysis of chitosan extracted from A. oryzae is presented in Figure 1. The analysis was undertaken to verify the specific absorption bands present in the recovered product [12]. The FTIR spectra of fungal chitosan exhibited conventional absorption bands of chitosan. The spectra displayed a broad absorption band at 3270.28 cm⁻¹ ascribed to the O-H stretching vibrations, indicative of CH₂OH groups [17]. The spectra also overlapped with N-H stretching vibrations of secondary amine.
The weak band at the absorption of 2915.75 cm\(^{-1}\) indicates the presence of O-H stretching (intramolecular bonded) vibrations. The presence of inter- and intramolecular hydrogen bonds stabilizes the structure of pure chitosan [18]. The sharpness of 2915.75 cm\(^{-1}\) and 2848.13 cm\(^{-1}\) corresponds to C-H stretching that suggested the presence of \(\alpha\)-polymorph crystallinity in the chitosan sample [17]. Chitin or chitosan can be composed of different polymorphs, which are \(\alpha\)- (antiparallel), \(\beta\)- (parallel) and \(\gamma\)- (two chains run in one direction and another chain runs in the opposite direction) polymorphs, that possess different molecular arrangement resulting in different crystal structures with dissimilar hydrogen bond densities [9, 19]. \(\alpha\)-chitin has strong intersheet and intrasheet hydrogen bonding, whereas \(\beta\)-chitin has a lower level of intermolecular hydrogen bonds than \(\alpha\)-chitin, as the \(\beta\)-chitin’s parallel molecular arrangement does not allow intersheet hydrogen bonds to occur [9, 19]. The increase of \(\alpha\)-polymorph of crystallinity may contribute to the increase of piezoelectric properties of chitin [17].

Medium band at the absorption of 1643.65 cm\(^{-1}\) and 1556.86 cm\(^{-1}\) attributed to N-H bending vibrations of amine and N-H deformation in CONH plane, including amide II, respectively [20]. The spectra at absorption of 1643.65 cm\(^{-1}\) also implies that most secondary amides were transformed into primary amines due to the deacetylation [20]. The medium band of infrared adsorption at 1410.28 cm\(^{-1}\) attributed to O-H bending vibration. The infrared absorptions by extracted chitosan at 1152.91 cm\(^{-1}\) and 1021.92 cm\(^{-1}\) were identified to be C-N stretching vibration of amine and C-O stretching vibration respectively.

3.3 Structural properties of fungal chitosan

X-ray diffraction (XRD) analysis was conducted on chitosan extracted from A. oryzae (Figure 2), which shows typical characteristic peaks of chitosan at 2\(\theta\) around 9-10\(^{\circ}\) and 19-20\(^{\circ}\) [21]. The XRD pattern shows peaks at 9.15\(^{\circ}\) due to chitosan sample being hydrated polymorphs of tendon form, in which bound water molecules incorporated into the crystal lattice [22, 23]. Bound water refers to ~10\% of water present in the chitosan sample even after the sample is freeze dried [22]. Small peak at 9.15\(^{\circ}\) also suggested the presence of anhydrous crystals [23]. The peak at 9.15\(^{\circ}\) represents diffraction intensity of amorphous region, whereas the peak at 19.60\(^{\circ}\) indicates diffraction intensity of crystalline region [20]. The peak at 19.60\(^{\circ}\) attributed
to the diffraction from the chitosan acetate crystal planes of (202) [24]. The broad hump at 19.60° suggested the semi-crystalline nature of chitosan sample [18].

![Figure 2 XRD analysis of chitosan from fungi A. oryzae](image)

Chitosan, a biological macromolecule composes of building blocks such as glucosamine, in which its structure is asymmetrical and it does not possess mirror reflection in nature [18]. Therefore, the most suitable space group for chitosan is P2₁2₁2₁, in which it is composed in cubic space groups of classes 23 and 432 from orthorhombic subgroups that are non-centrosymmetric [18]. The piezoelectric properties of chitin were reported to be ascribed to intrinsic molecular polarization arising from the non-centrosymmetric crystal structure of chitin polymorphs [3].

FESEM was undertaken in order to analyze the surface morphology of chitosan (Figure 3). FESEM analysis exhibited that chitosan sample had a long thin crystal structure on a smooth surface [13].
4. Conclusion

Chitosan could be extracted from fungal biomass of *Aspergillus oryzae* as a sustainable source for piezoelectric material. The structural analysis of the microbial-derived chitosan showed that it has potential to be used in piezoelectric application due to the presence of non-centrosymmetric crystal structure.

Acknowledgement

This work was supported by Research Initiative Grant Scheme (RIGS) 2017 of International Islamic University Malaysia (Project ID: RIGS17-013-0588). The authors would like to thank Degan Barkadleh Youssouf for research assistantship.

Reference

[1] Katsuura T, Izumi S, Yoshimoto M, Kawaguchi H, Yoshimoto S and Sekitani T 2017 Wearable pulse wave velocity sensor using flexible piezoelectric film array. In: 2017 IEEE Biomedical Circuits and Systems Conference (BioCAS): IEEE) pp 1-4

[2] Jacob J, More N, Kalia K and Kapusetti G 2018 Piezoelectric smart biomaterials for bone and cartilage tissue engineering Inflammation and Regeneration 38 2

[3] Kim K, Ha M, Choi B, Joo S H, Kang H S, Park J H, Gu B, Park C, Park C and Kim J 2018 Biodegradable, electro-active chitin nanofiber films for flexible piezoelectric transducers Nano Energy 48 275-83

[4] Akmal M H M, Warikh A R M, Azlan U A A, Azmi N A, Salleh M S and Kasim M S 2018 Optimizing the processing conditions of sodium potassium niobate thin films prepared by sol-gel spin coating technique Ceramics International 44 317-25

[5] Hänninen A, Sarlin E, Lyyra I, Salpavaara T, Kellomäki M and Tuukkanen S 2018 Nanocellulose and chitosan based films as low cost, green piezoelectric materials Carbohydrate polymers 202 418-24
[6] Maghsoudi V, Razavi J and Yaghmaei S 2009 Production of chitosan by submerged fermentation from Aspergillus niger *Scientia Iranica. Transaction C, Chemistry, Chemical Engineering* **16** 145

[7] Hoque N A, Thakur P, Biswas P, Saikh M M, Roy S, Bagchi B, Das S and Ray P P 2018 Biowaste crab shell-extracted chitin nanofiber-based superior piezoelectric nanogenerator *Journal of Materials Chemistry A* **6** 13848-58

[8] Chen Y, Liu Z, Li M, Wu X, You J and Li C 2017 Guiding growth orientation of two-dimensional Au nanocrystals with marine chitin nanofibrils for ultrasensitive and ultrafast sensing hybrids *Journal of Materials Chemistry B* **6** 13848-58

[9] Jin J, Lee D, Im H-G, Han Y C, Jeong E G, Rolandi M, Choi K C and Bae B-S 2016 Chitin Nanofiber Transparent Paper for Flexible Green Electronics *Advanced Materials* **28** 5169-75

[10] Hänninen A, Rajala S, Salpavaara T, Kellomäki M and Tuukkanen S 2016 Piezoelectric Sensitivity of a Layered Film of Chitosan and Cellulose Nanocrystals *Procedia Engineering* **168** 1176-9

[11] Zamani A, Edebo L, Sjöström B and Taherzadeh M J 2007 Extraction and precipitation of chitosan from cell wall of zygomycetes fungi by dilute sulfuric acid *Biomacromolecules* **8** 3786-90

[12] Abdel-Gawad K M, Hifney A F, Fawzy M A and Gomaa M 2017 Technology optimization of chitosan production from Aspergillus niger biomass and its functional activities *Food Hydrocolloids* **63** 593-601

[13] Johney J, Eagogpan K and Ragunathan R 2016 Microbial extraction of chitin and chitosan from Pleurotus spp, its characterization and antimicrobial activity *Int J Curr Pharm Res* **9** 88-93

[14] Ahmad F B, Zhang Z, Doherty W O S and O'Hara I M 2016 Evaluation of oil production from oil palm empty fruit bunch by oleaginous micro-organisms *Biofuels, Bioproducts and Biorefining* **10** 378-92

[15] Horikoshi K and Iida S 1964 Studies of the spore coats of fungi I. Isolation and composition of the spore coats of Aspergillus oryzae *Biochimica et Biophysica Acta (BBA) - Specialized Section on Mucoproteins and Mucopolysaccharides* **83** 197-203

[16] Rane K D and Hoover D G 1993 An evaluation of alkali and acid treatments for chitosan extraction from fungi *Process Biochemistry* **28** 115-8

[17] Street R M, Husseynova T, Xu X, Chandrasekaran P, Han L, Shih W Y, Shih W-H and Schauer C L 2018 Variable piezoelectricity of electrospun chitin *Carbohydrate Polymers* **195** 218-24

[18] Praveen E, Murugan S and Jayakumar K 2017 Investigations on the existence of piezoelectric property of a bio-polymer – chitosan and its application in vibration sensors *Rsc Adv* **7** 35490-5

[19] Jang M-K, Kong B-G, Jeong Y-I, Lee C H and Nah J-W 2004 Physicochemical characterization of α-chitin, β-chitin, and γ-chitin separated from natural resources *Journal of Polymer Science Part A: Polymer Chemistry* **42** 3423-32

[20] Berger L R R, Stamford T C M, de Oliveira K A R, Pessoa A d M P, de Lima M A B, Pintado M M E, Câmara M P S, de Oliveira Franco L, Magnani M and de Souza E L 2018 Chitosan produced from Mucorales fungi using agroindustrial by-products and its efficacy to inhibit Colletotrichum species *International journal of biological macromolecules* **108** 635-41

[21] Jaworska M, Sakurai K, Gaudon P and Guibal E 2003 Influence of chitosan characteristics on polymer properties. I: Crystallographic properties *Polymer International* **52** 198-205
[22] Prashanth K H, Kittur F and Tharanathan R 2002 Solid state structure of chitosan prepared under different N-deacetylating conditions *Carbohydrate Polymers* **50** 27-33

[23] Ogawa K 1991 Effect of heating an aqueous suspension of chitosan on the crystallinity and polymorphs *Agricultural and biological chemistry* **55** 2375-9

[24] Zhao J, Han W, Chen H, Tu M, Zeng R, Shi Y, Cha Z and Zhou C 2011 Preparation, structure and crystallinity of chitosan nano-fibers by a solid–liquid phase separation technique *Carbohydrate polymers* **83** 1541-6