Microbial chitosan for the fabrication of piezoelectric thin film

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Abstract. Chitin has proven to have a good mechanical and electrical properties to be used in making piezoelectric thin films. However, due to the restriction in solubilizing chitosan in many solvents, there is increasing interest in exploring the used of chitosan in producing thin films. Chitosan, compared to chitin, can be easily solubilized in certain dilute acids. Chitosan that has been extracted from fungal biomass can be used for the fabrication of biomaterial thin films. There are different ways that can be used to fabricate a thin film such as electrospinning, spin-coating, solvent casting and also the hot press technique.

Keywords: Chitosan; chitin; electrospinning; fungi; piezoelectric; thin films

1. Introduction
Chitin is the second most abundant natural polysaccharide that can be extracted from the shells of crab, shrimp, and prawn [1]. It is a linear 1,4-linked polymer that is made up of N-acetyl-D-glucosamine residues [2]. As chitin is a semi-crystalline polymer with high hydrogen bond, the solubility of the chitin in most organic solvents is not feasible [3].

There are two allomorphs of the chitin which are α-chitin and β-chitin [4]. The most abundant one is the α-chitin where it can be found in the cell wall of yeast and fungi, krill, lobster, crab and shrimp whereas the β-chitin is the rare type, naturally occurring in squid pens, vestimetiferan worms and pogonohoran [5]. Both allomorphs of chitin are insoluble in most solvents making it one of the challenges for it [5]. Only few solvents that can solubilize chitin are hexafluoro-isopropanol, hexafluoroacetone, chloroalcohols and dimethylacetamide, that are typically toxic and dangerous to nature [6].
Chitosan can be produced from chitin through process called deacetylation. Compared to chitin, chitosan can be solubilized in dilute acids. The solubility of this material depends on the types (mineral or organic) and concentration of the acids used [3]. One of the main sources of chitin is from the crustacean shells where annually it has been produced around 1.2 mega tons [7]. As there are limitations in the supply of this material due to season and limitation in the fishing industry itself, fungal biomass-derived chitin and chitosan has gained more attention [8]. This study reviews various methods for extraction of chitosan from fungal biomass and fabrication of thin film from chitosan.

2. Chitin isolation and chitosan extraction from microbial biomass
Crustacean-based chitin extraction involves three processes involving demineralization, deproteinization and deacetylation [9]. In demineralization step, the inorganic calcium carbonate is removed while the protein is removed during deproteinization. The demineralization and deproteinization processes can be performed either using chemical treatments or enzymatic treatments [5].

The use of fungi as the source of chitin and chitosan is promising due to the sustainability of fungal cultivation that is not limited by the supply from fishing industry [10]. Fungal biomass is acquired from the harvesting of fungal cell at the end of cultivation. Fungi can be cultivated in large scale bioreactor, in which large scale of production of fungal biomass does not requires large area of available land [11]. The pathway of extraction of chitin from fungi biomass is similar to that from crustacean. However, the chitin extracted from fungal biomass does not require demineralization process [8].

The first step in converting chitin to chitosan is the deproteinization as the chitin has a high protein content [10]. This step is said to be difficult to disrupt the bond between chitin and protein. The most preferred reagent used by many studies is NaOH [5].

The next important step is the removal of B-glucan from fungal biomass. In fungal cell wall, the major polysaccharide existed is the B-glucan which consists of (1→3) backbone with (1→6) branches [12]. Most of the glucan int the biomass can be degraded with the help of harsh acid treatment [4].

During the process of deacetylation of chitin, chitosan is produced by removing the acetamide group in chitin and replacing with amino group [13]. Figure 1 shows the chemical structure for deacetylation of chitin where chitosan will be produced at the end of the reaction.

![Chemical structure of chitin and chitosan](image)

**Figure 1.** The chemical structure of chitin and chitosan in deacetylation process.

Table 1 shows several methods of extraction of chitosan from fungal biomass and chitosan yield obtained through respective methods. Although most of the methods for the extraction were using the alkaline treatment, method developed by Zamani et al. involved the application of dilute sulfuric acid treatment. In the extraction method from that study, chitosan was being recovered by precipitation at lower temperature after treating fungal biomass with strong acid [14]. There are studies that used enzymatic reaction in extracting chitosan from fungal biomass, where the α-amylase was used [15]. The enzyme used in the study help to breakdown the chitosan-glucan complex and produce a better quality of chitosan [15]. However, the enzymatic extraction method was time-consuming and costly.
Table 1. Methods of chitin isolation and chitosan extraction, chitosan yield and the degree of deacetylation (DDA).

| Fungal strain                  | Method of extraction | Chitosan yield (%) | DDA (%) | Ref. |
|--------------------------------|----------------------|--------------------|---------|------|
| Pre-treatment                  | Extraction           |                    |         |      |
| *Rhizomucor pusillus*          | Alkaline treatment using NaOH 0.5 M at 120 °C for 20 min. AIM was treated with 72 mM sulfuric acid at 22 °C for 10 min and proceed with same treatment for another 45 min. | 28.7 | - | [14] |
| *Gongronella butleri*          | Biomass was dried at 45 °C | Treated with 10 mL of 1 M NaOH per g of mycelia at 40 °C for 16 h. AIM was washed and dried before subjected to 200 mL of 0.35 M of acetic acid at 40 °C for 16 h. | 1.9 | 13 | [15] |
|                                |                      | Treated with 10 mL of 11 M NaOH containing 0.05 g of sodium borohydride per g of mycelia (40 °C, 13 h). AIM washed until neutral pH and dried before subjected to 200 mL of 0.35 M acetic acid at 95 °C for 5 h. pH of AIM was adjusted to 4.5 using 1 M NaOH and 4% (v/v) α-amylase and incubated in water bath shaker at 65 °C, 200 rpm for 3 h. | 5.9 | 13 |      |
| *Penicillium chrysogenum*      | Dry biomass was pulverized | Alkaline treatment using 1 M NaOH for 1:30 (w/v) and autoclaved at 121 °C for 15 min. | 12.2 | 82.4 | [16] |
| *Aspergillus niger*            | Dry biomass was milled with blender | Alkaline treatment using 1 M NaOH for 1:40 (w/v) at 90 °C for 3 h. AIM was treated with 10% acetic acid at 1:40 (w/v) at room temperature for 6 h on 200 rpm shaker. | 7.57 | 83.64 | [17] |
| *Mucor genevensis* (chitin-chitosan thin film) | Biomass blended for 5 min with water | Alkaline treatment using 1 M NaOH at 65 °C for 3 h, cooled and neutralised | - | Partially deacetylated | [18] |
3. Method to fabricate chitosan thin film

One of the biodegradable natural polymers is chitosan. The degree of deacetylation of the chitin should reach about 50% to ensure that the chitins were successfully converted into chitosan [1]. Chitosan thin film has various application including for piezoelectric application [1]. The properties of chitosan are biocompatibility, biodegradability, non-toxic and can be easily formed as film, making it suitable to be used in piezoelectric application. Thin film fabricated using pure chitosan can produce higher piezoelectricity compared to thin film from composite materials [6]. However, there are issues concerning the process of fabrication of chitosan thin film as it only dissolves in polar acidic solutions [19]. There are several methods in fabrication the chitosan thin film such as electrospinning, spin coating and also solvent casting. To date, all studies on chitosan thin film for piezoelectric application were focusing on the use of solvent casting [1,20].

3.1. Electrospinning

Electrospinning is one of the process used to synthesis polymer fibres that utilizes high voltage to develop electrostatic force. As thin films are widely explored, electrospinning has been one of the attractions in film fabrication as it can synthesize material customized for diameters ranging between nanometers and micrometers. The basic setup of this method, as shown in figure 2, consists of injection pump, power supply, fixture that support the spinneret and plate [21]. Chitosan has difficulty to solubilize in aqueous and organic solution as it has high crystallinity properties. Due to this, chitosan is mixed with other polymer to help with the spinnability of chitosan. It has been reported that medium and high molecular weight of chitosan will produce a uniform surface using electrospinning [3]. The drawback of this method is the formation of beads along with the fibers [20].

![Figure 2. The basic setup of electrospinning.](image)

3.2. Spin-coating

Spin-coating is a method used to make a thin film that is uniform and the thickness can be in nanoscale [19]. There are five stages of spin coating which are solution deposition, acceleration, viscosity-controlled thinning, evaporation-controlled thinning and lastly drying [22]. As the solution is deposited on a substrate, the disc will be rotated until it reaches the desired speed. The common speed used in spin coating are between 2000 to 6000 rpm [22]. The centrifugal forces and adhesion forces from the solution-substrate will create strong sheering that leads to radial flow and ejection of the solution from the substrate, resulting in a uniform and thin chitosan film [19]. With this method, the thickness of the film created can be controlled easily by changing the spin speed on the substrate. Figure 3 shows the setup of spin-coating method. However, as the size of the substrate increases, the thinning of the film becomes hard as it is harder to control the high-speed spinning of the disc [23].
3.3. Solvent casting

The most basic method for fabricating thin film is the solvent casting method. The polymer needs to be soluble in water or volatile solvent before doing the casting. It is also important that the mixture need to have minimum solid content and viscosity and the material used must be able to form homogeneous film and be separated from the casting surface [24]. The drawback from this method is that it cannot produce very thin films from polymers without stretching the film during drying and the films produced are more hydrophilic compared to electrospun films [24]. Figure 4 shows the solvent casting process of thin film fabrication.

![Figure 3. The setup of spin-coating method in fabricating thin film.](image)

3.4. Hot press technique

This technique is not a common choice as it is hard to get a low thickness film. However, a study by Hashizume et al. reported on fabrication of chitosan thin film using hot press. The main point of this technique is that the mixture of chitosan needs to be freeze dry prior to hot press [25]. During the process, polyethylene terephthalate (PET) sheets and polytetrafluoroethylene (PTFE) sheets were used as separator between the sample and hot press stage in order to avoid contamination of the thin film. In the

![Figure 4. The solvent casting for thin film.](image)
study by Hashizume et al., the temperature and pressure used for the hot press technique were set at 120 °C and 20 MPa respectively [25].

4. Conclusions
The production of chitosan from microbial biomass is attractive due to the sustainable supply chain. There are many ways to successfully extract chitosan from microbial biomass. The fabrication of chitosan into thin film is crucial for piezoelectric application. The use of chitosan in piezoelectric application is significant as chitosan is a biomaterial that is biocompatible which enable its application as biomedical piezoelectric devices. All of the methods of fabricating the thin films mentioned before has proved to successfully producing a good quality of chitosan thin film.

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