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Seafood Processing Chitin Waste for Electricity Generation in a Microbial Fuel Cell Using Halotolerant Catalyst *Oceanisphaera arctica* YHY1

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Abstract: In this study, a newly isolated halotolerant strain *Oceanisphaera arctica* YHY1, capable of hydrolyzing seafood processing waste chitin biomass, is reported. Microbial fuel cells fed with 1% chitin and 40 g L⁻¹ as the optimum salt concentration demonstrated stable electricity generation until 216 h (0.228 mA/cm²). N-acetyl-D-glucosamine (GlcNAc) was the main by-product in the chitin degradation, reaching a maximum concentration of 192.01 mg g⁻¹ chitin at 120 h, whereas lactate, acetate, propionate, and butyrate were the major metabolites detected in the chitin degradation. *O. arctica* YHY1 utilized the produced GlcNAc, lactate, acetate, and propionate as the electron donors to generate the electric current. Cyclic voltammetry (CV) investigation revealed the participation of outer membrane-bound cytochromes, with extracellular redox mediators partly involved in the electron transfer mechanism. Furthermore, the changes in structural and functional groups in chitin after degradation were analyzed using FTIR and XRD. Therefore, the ability of *O. arctica* YHY1 to utilize waste chitin biomass under high salinities can be explored to treat seafood processing brine or high salt wastewater containing chitin with concurrent electricity generation.

Keywords: chitin; electricity generation; halotolerant; microbial fuel cell; seafood processing

1. Introduction

The seafood processing industry produces a substantial amount of wastewater, mainly containing soluble, colloidal, and particulate matter. Among crustaceans, the total production of shrimp reached 5.03 million tons in 2020 and is estimated to increase to 7.28 million tons by 2025, with a compound annual growth rate of 6.1% from 2020 to 2025 and 67.6 billion USD turnover [1]. Asia alone contributes more than 80% of the global shrimp production, where Thailand is the major exporter of cultivated shrimp to the USA, Canada, Europe, South Korea, and Japan [2]. Depending on the market requirements, shrimp is exported or stored in frozen conditions with or without an outer shell. For shrimp processing, a huge amount of water is required, generating around 1000 L of highly polluted wastewater per ton of shrimp [1,3]. Shrimp processing produces 50–60% of solid waste comprising the head, viscera, and shell, which are discarded as the by-products generated in processing. The biochemical composition of shrimp waste mainly contains 15–46% chitin, 30–60% minerals, 10–40% protein, and 10–40% lipids [1,4]. Similarly, the salinity of the seafood processing wastewater is another important factor that mainly depends on the products or species being processed. The precooking or

brine treatment for the canning of shrimp generates wastewater, with the NaCl concentration ranging between 20–30 g L⁻¹ [5].

Chitin is the second most abundant natural biopolymer, consisting of monomeric units of GlcNAc linked with (1,4)- β -linkages [6]. The microbial chitinase (EC 3.2.2.14) is a glycoside hydrolytic enzyme capable of hydrolyzing chitin into GlcNAc or oligomers and utilizing it as a source of carbon for growth and development [7]. Chitin waste has been partly used as animal feed, as a component of aquaculture feed formulation, and for the recovery of bioactive molecules. However, a large amount of chitin biomass is being wasted, which increases environmental pollution [1]. However, particulate substrates like chitin can be inexpensive, easily available, and renewable feedstock in microbial fuel cells (MFCs) for electricity production [8]. The MFC is a bio-electrochemical system that can break and utilize chitin more effectively compared to normal fermentation conditions, owing to the anode working as an electron acceptor [7,8]. Several natural and synthetic substrates, including acetate, glucose, lactate, amino acids, butyrate, formate, fumarate, alcohols, or complex carbohydrates like cellulose, sucrose, molasses, starch, or industrial and domestic wastewater, were widely researched as fuels for MFCs [8,9]. However, high production costs and easy depletion have constrained the use of synthetic substrates in MFCs. Nevertheless, chitin waste can be an alternative synthetic substrate source for sustainable energy production by using bacteria as a catalyst to oxidize organic substrates directly into electrical energy. Previously, chitin has been utilized as a substrate by *Bacillus circulans*, *Arenibacter palladensis*, *Shewanella oneidensis*, *Aeromonas hydrophila*, or as sediment wastewater-based systems for electricity production [7,10].

Therefore, in the present study, a newly isolated halotolerant marine bacterium *O. arctica* YHY1 capable of hydrolyzing chitin waste was studied as a biocatalyst for electricity generation in MFCs. Furthermore, the electrochemical parameters, the structural changes in chitin before and after degradation, and the by-products or metabolites of degradation were analyzed.

2. Materials and Methods

2.1. Chitin Preparation, Isolation, and Identification of Chitin Degrading Microbes

The shrimp shell chitin was obtained from the local seafood processing unit near Seoul, South Korea. The obtained chitin biomass was washed, dried, and pre-treated according to the method reported earlier by Gurav et al. [10]. The resulting colloidal chitin was dried and stored in a refrigerator for further applications. Isolation of chitinolytic microbes was performed using marine soil collected from a beach near Geoje (34°51'16.5'' N 128°43'43.9'' E), Eastern Sea of South Korea. In brief, one gram of soil was serially diluted and smeared on the chitin agar plates containing (g L⁻¹) 0.7-KH₂PO₄, 0.3-K₂HPO₄, 5.0-NaCl, 0.5-MgSO₄, 0.0001-ZnSO₄, 0.0001-MnSO₄, 10-chitin, and 25-agar [6,10]. Seven morphologically different strains showing clear zones were isolated and cultured as a monoculture on chitin-agar plates. These seven strains were initially tested for electric current production in MFCs supplemented with 1% chitin. Based on its higher chitin degradation and electricity generation ability, strain YHY1 was selected for identification by using 16S rRNA gene sequencing.

2.2. MFC Setup and Electrochemical Analysis

As shown in Figure 1, a dual-chamber MFC consisting of cathodic and anodic chambers separated by a proton exchange membrane (Nafion 212, Omniscience, Yongin, Korea) was assembled. The anodic chamber was equipped with 2.25 cm² carbon felt and a silver reference electrode (Ag/AgCl), whereas the cathodic chamber contained 4 cm² platinum-coated carbon felt. Degassed growth media containing (g L⁻¹) 0.7-KH₂PO₄, 0.3-K₂HPO₄, 40-NaCl, 0.5-MgSO₄, 0.0001-ZnSO₄, 0.0001-MnSO₄, and 10-chitin was inoculated with 1% *v/v* inoculum with an optical density of 0.9 ± 0.05 at the anodic chamber, whereas 50 mL phosphate buffer (pH 7.0; 50 mM) containing 50 mM ferricyanide was filled at the cathode chamber. The anode and cathode were connected through a potentiostat (WizECM-8100

premium, Wizmac, Daejeon, Korea) and operated as a closed-circuit using an external resistance of 1000Ω [7,8,11,12]. The current output density (mA/cm^2) of the system was recorded and plotted versus time (h). To investigate the electrocatalytic behavior and interaction between the redox mediator, electrode, and anodic biofilm, the CV was performed using a three-electrode system including working, counter, and reference electrodes with a $10 \text{ mV}/\text{S}$ scan rate and a $+1 \text{ V}$ to -1 V potential range.

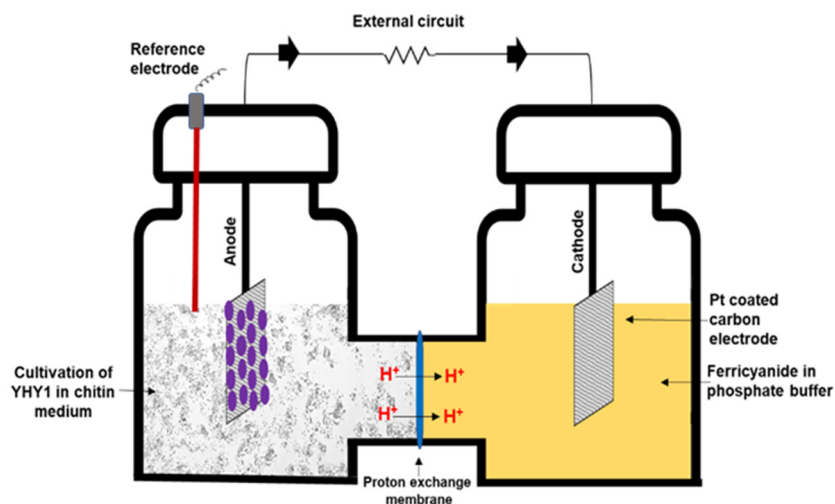


Figure 1. Setup of the dual-chamber MFC.

2.3. Analysis of Degradation Products

HPLC (Young Lin, YL-9100, Seoul, Korea) investigation was performed to quantify free GlcNAc in the degradation media using a C18 column (ZORBAX, SBC18) and acetonitrile:water (20:80) as the mobile phase [7,10]. Further, the metabolic profiling of the chitin degradation media was performed using HPLC (Bio-Rad, Hercules, CA, USA) equipped with Bio-Rad Aminex HPX-87H column and $5 \text{ mM H}_2\text{SO}_4$ as the solvent phase [13].

2.4. SEM, XRD and FTIR Analysis

SEM (Hitachi TM4000Plus, Tokyo, Japan) was performed to investigate the anodic biofilm [9]. The FTIR (Nicolet 6700, Thermo Fisher Scientific, Waltham, MA, USA) was executed to verify the structural changes in the chitin before and after degradation in MFCs [7,10], whereas XRD (D8 ADVANCE-DAVINCI, Bruker, Bremen, Germany) was examined to determine changes in the crystallinity of chitin. The crystalline index (CrI , %) was calculated from the XRD data using the following equations [10,14].

$$\text{CrI}_{020} = [(I_{020} - I_{\text{am}})/I_{020} \times 100] \quad (1)$$

where I_{020} and I_{am} is the maximum intensity at $2\theta \cong 9^\circ$ and the intensity of amorphous diffraction at $2\theta \cong 16^\circ$, respectively.

$$\text{CrI}_{110} = [(I_{110} - I_{\text{am}})/I_{110} \times 100] \quad (2)$$

where I_{110} is the maximum intensity at $2\theta \cong 20^\circ$.

3. Results

3.1. Identification, and Salt-Tolerance in the Chitinolytic Bacterium

Seven chitin degrading strains were newly isolated from the marine soil, where strain YHY1 showed maximum chitin hydrolysis activity with a zone diameter of 20 mm on chitin agar plates. The 16S rRNA sequencing data of strain YHY1 revealed 96% similarity with *Oceanisphaera arctica* strain V1-41; therefore, strain YHY1 was designated as *Oceanisphaera arctica* YHY1. The phylogenetic position of strain YHY1 and other allied strains is depicted

in Figure 2a. The obtained gene sequence (989 bp) was submitted to Genbank under accession no. MH590704. Furthermore, the salt tolerance in strain YHY1 showed better growth on increasing the salt concentration from 5 g L⁻¹ to 40 g L⁻¹, suggesting the necessity of salt for growth (Figure 2b). This strain was able to tolerate up to 80 g L⁻¹ of salt concentration; however, concentrations above 80 g L⁻¹ significantly inhibited the growth of the bacterium.

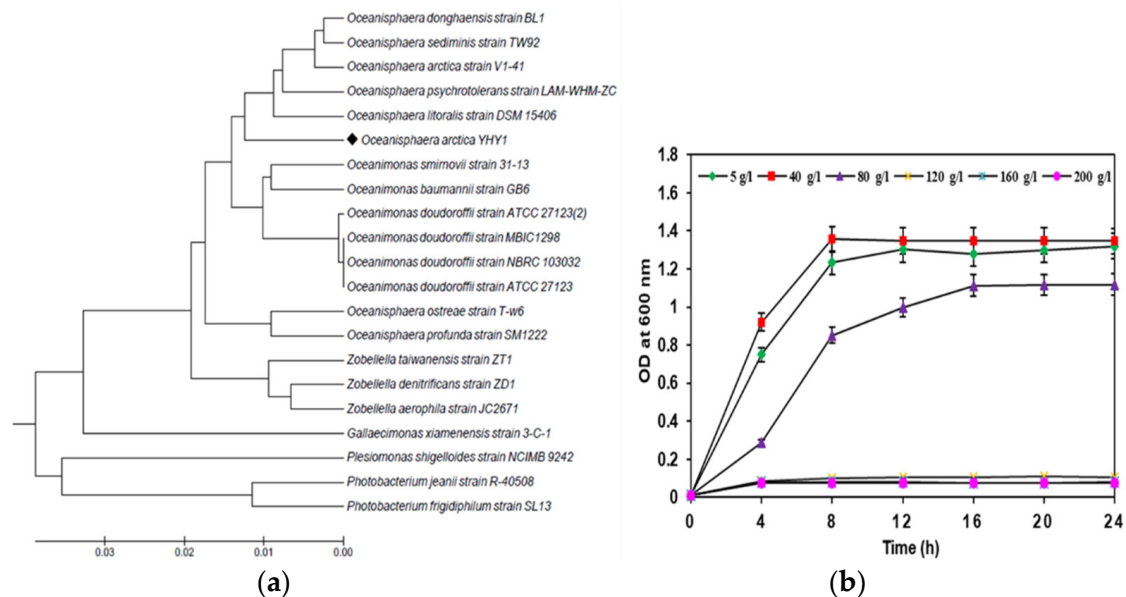


Figure 2. (a) Phylogenetic position of *O. arctica* YHY1; (b) Salt tolerance in strain YHY1.

3.2. Electrochemical Assessment of MFCs Fueled with Chitin Waste

Depleting non-renewable energy resources has enabled researchers to discover new sustainable energy assets. In MFCs, microbes act as the biocatalysts that transform the chemical energy of organic residues into electricity [10,15]. In this study, halotolerant *O. arctica* YHY1 was investigated for electricity production using seafood processing waste chitin as the carbon source. As shown in Figure 3a, the performance of MFCs fueled with 1% chitin biomass was studied, with a fixed external resistance of 1000 Ω and 40 g L⁻¹ NaCl. After inoculating the MFCs with *O. arctica* YHY1, a rapid increase in electricity generation was observed in the MFCs, with a maximum current output density reaching 0.302 mA/cm² at 12 h. Higher current production in the initial period could be related to the free GlcNAc (12.311 mg g⁻¹ chitin) that was produced during the pre-treatment of the chitin. A similar mechanism has been reported earlier in *Aeromonas hydrophila*, *Bacillus circulans*, and *Shewanella oneidensis* MFCs using chitin as the carbon source [7,11,12]. However, the current output density slightly dropped to 0.210 mA/cm² after 20 h; this might be due to depletion in free GlcNAc. Thereafter, the electricity generation was enhanced and remained almost constant until 216 h (0.228 mA/cm²). Previously, chitin or pure GlcNAc has been reported as an electron donor in electricity production in MFCs using various microbial catalysts. For instance, *Aeromonas hydrophila* and *Shewanella oneidensis* fed with 0.2% chitin produced 8.77 μ A/cm² and 4.24 μ A/cm², whereas using pure GlcNAc produced 6.65 μ A/cm² and 6.17 μ A/cm² of current output density, respectively [11,12]. Similarly, *Arenibacter palladensis* and *Bacillus circulans* produced 15.15 μ A/cm² and 26.508 μ A/cm² current output density, respectively, in MFCs fed with 1% chitin [7,10]. However, in the present study, a stable and higher current output density of 0.228 mA/cm² (216 h) was observed in *O. arctica* YHY1 using 1% chitin and 40 g L⁻¹ salt concentration. Salinity played an important role in the MFCs in the present study. The MFCs were supplied with 40 g L⁻¹ NaCl as the optimized salt concentration for *O. arctica* YHY1. The salinity of the media has a positive impact on electricity generation

as it decreases the internal resistance of the system and increases media conductivity. Furthermore, higher salinity can also prevent the acidification of the media due to the fast transfer of H^+ ions from the anode to the cathode [9,16].

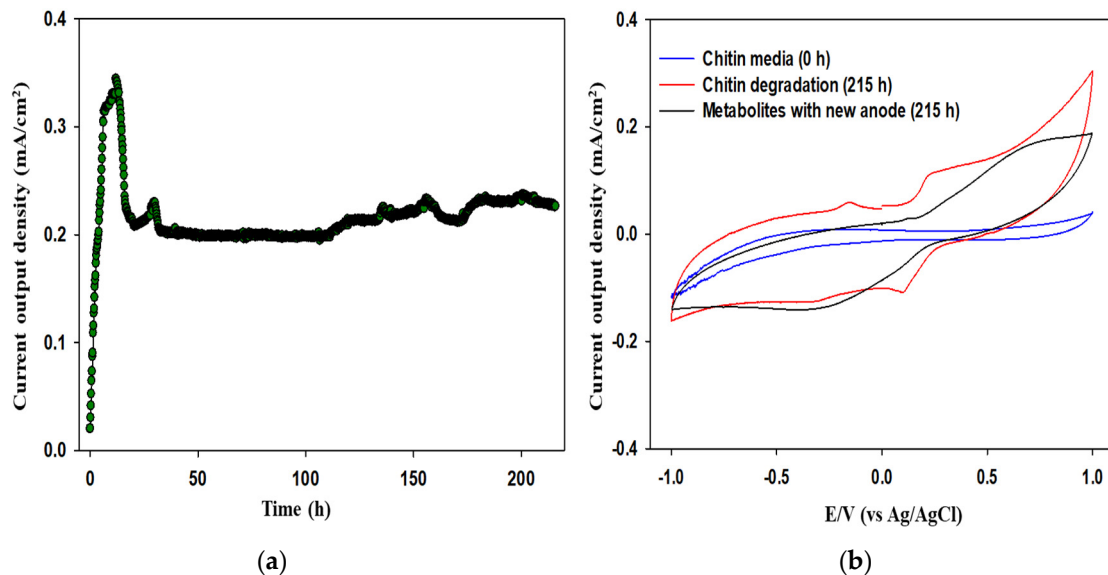


Figure 3. (a) Performance of strain YHY1 in MFCs fed with 1% chitin, 40 g L^{-1} NaCl, and external load of 1000Ω ; (b) CV of the MFCs fed with chitin. Plots represent the CV of media without inoculum (0 h), CV of media at 216 h of growth, CV of cell-free metabolites with a new anode.

CV studies were executed to reveal the electrochemical behavior of *O. arctica* YHY1 in MFCs fed with chitin. As depicted in Figure 3b, the initial CV profile of chitin media (0 h) without bacterial inoculation did not show any oxidation-reduction peaks, indicating a lack of redox mediators in the medium. However, the CV of MFCs at 216 h after inoculation showed distinct oxidation peaks at -0.15 V (vs. Ag/AgCl), and $+0.20 \text{ V}$ (vs. Ag/AgCl), reduction peaks at $+0.10 \text{ V}$ (vs. Ag/AgCl), and a broad peak at -0.40 V (vs. Ag/AgCl) (Figure 3b). During the forward scan, a higher current output density of 0.302 mA/cm^2 was observed, indicating higher oxidation reactions were recorded, as compared to the reductions reported earlier [7,17]. The CV of the cell-free degradation metabolites after the filtration and insertion of a new anode showed a broad oxidation-reduction peak at $+,-0.40 \text{ V}$ (vs. Ag/AgCl), suggesting production of a low quantity of soluble redox mediators in the chitin degraded medium. The observed formal potential of 0.200 V (vs. Ag/AgCl) can be related to the outer membrane-bound cytochrome, which can transfer the electrons directly to the electrode without any external redox mediators, as reported earlier in *Shewanella* [18]. Further, a formal potential of -0.15 V (vs. Ag/AgCl) was detected similar to *Geobacter sulfurreducens*, which generally use omcB to transfer electrons through the electrode/biofilm interface [19–22]. Similarly, the low amount of flavins might have been produced by *O. arctica* YHY1 with the formal potential of 0.40 V (vs. Ag/AgCl), as reported earlier [23]. Therefore, from the CV data, it could be predicted that *O. arctica* YHY1 mainly utilizes a direct electron transfer pathway to transfer electrons directly to the electrode surface using membrane-bound cytochromes. Furthermore, this bacterium also partly employs the indirect electron transfer pathway using extracellular redox mediators to shuttle electrons to the electrode. However, more detailed study at a molecular level is needed to find the exact mechanism used by *O. arctica* YHY1 to transfer electrons from bacteria to the electrode surface.

3.3. Chitin Degradation By-Products and Other Metabolites

Chitinolytic microbes can hydrolyze chitin biopolymer into monomeric or dimeric GlcNAc units and utilize them as the source of energy. In the present study, monomeric

GlcNAc was the main by-product of chitin hydrolysis by *O. arctica* YHY1 in MFCs. The concentration of GlcNAc at 24 h was 58.21 mg g^{-1} chitin, which was significantly increased to 192.01 mg g^{-1} at 120 h. However, after 120 h, the concentration of GlcNAc was gradually decreased to 76.22 mg g^{-1} at 216 h. From these results, it could be concluded that until 120 h, chitin was efficiently degraded, with simultaneous utilization of GlcNAc. Thereafter, depletion in the chitin concentration in media might have terminated the chitinase-producing machinery or inhibited the enzyme activity due to the generation of toxic intermediate metabolites [7,24]. However, *O. arctica* YHY1 continued to utilize the produced GlcNAc with the concurrent electricity generation until 216 h. During hydrolysis of the chitin, several metabolites were detected in the MFCs, with lactate, acetate, propionate, and butyrate as the prominent metabolites. The highest concentration of acetate, 5.901 mM (144 h), was detected, followed by butyrate, 3.572 mM (96 h), lactate, 0.932 mM (96 h), and propionate, 0.157 (120 h) (Figure 4a). Acetate, lactate, and propionate were most preferred by *O. arctica* YHY1, with 1.593 mM , 0.110 mM , and 0.099 mM concentrations, respectively, remaining unutilized at 216 h. However, butyrate was strain YHY1's less favored electron donor (2.533 mM ; 216 h). Although the GlcNAc content was depleted after 120 h of growth, the current output density remained stable as the bacterium also utilized produced metabolites like lactate and acetate for growth, which correlates with the previous report on chitin as the carbon source in MFCs [7]. In *Shewanella*, lactate is first produced, followed by pyruvate, and acetate, and thus the carbon source preference can be in the following order: lactate \rightarrow pyruvate \rightarrow acetate [25]. The lactate concentration was higher at 96 h whereas acetate was higher at 144 h, although both concentrations decreased after reaching the maximum concentration, suggesting lactate can be oxidized to acetate [26]. During chitin hydrolysis, the production of metabolites like lactate, acetate, butyrate, succinate, format, and propionate was reported earlier in *Shewanella oneidensis*, *Bacillus circulans*, *Arenibacter palladensis*, and *Aeromonas hydrophila* [7,10–12].

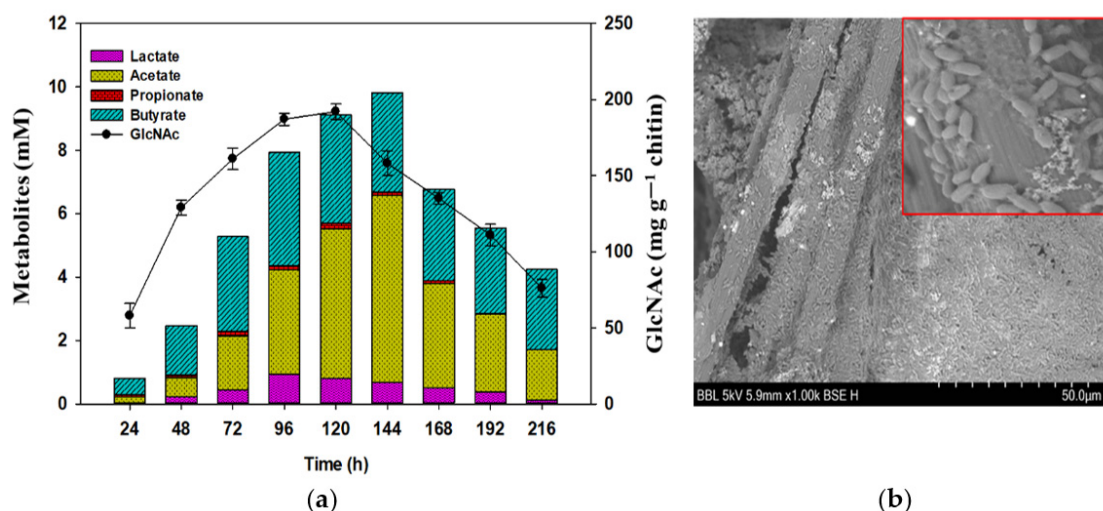


Figure 4. (a) Production and utilization of GlcNAc and other metabolites in MFC; (b) SEM examination of anodic biofilm.

SEM investigation of the anodic biofilm of *O. arctica* YHY1 revealed a thick biofilm formation on the anode surface (Figure 4b). This result could also be correlated with the CV data, suggesting that membrane-bound cytochromes were principally involved in direct electron transfer to the anode.

3.4. Investigating Structural Changes in Chitin Polymer in MFCs

As shown in Figure 5a, chitin biopolymer has a typical band pattern for amide bonds at 1660 and 1630 cm^{-1} (amide I), 1558 cm^{-1} (amide II), 1318 cm^{-1} (amide III), and 693 cm^{-1} (amide V) [7,27]. Similarly, an IR peak at 1028 cm^{-1} assigned to stretching vibration for —C—O—C of the glucosamine ring, and a peak at 890 cm^{-1} related to ring stretching for

β -1,4 glycosidic bonds were detected in the chitin [28]. Nevertheless, on the degradation of chitin in MFCs, the band intensity at 1660 cm^{-1} (amide I), 1378 cm^{-1} (amide III), and 1080 cm^{-1} (C–O stretching) substantially decreased, suggesting breaking of the C–O and C–H bonds [7,10]. Likewise, the XRD analysis revealed alterations in the crystallinity of the chitin before and after degradation (Figure 5b). The diffraction pattern of chitin before degradation showed distinct peaks at lattice (020), (110), (120), (101), and (130) [7]. Strong reflections were detected at 2θ 9.45° , 19.05° , and 31.46° , whereas other peaks were detected at 12.93° , 20.09° , 23.60° , and 26.51° . The crystalline index (CrI) of the chitin before and after degradation was calculated considering reflections at (020) and (110). Initial CrI(020) and CrI(110) of the chitin were 74.41% and 83.60%, respectively, which significantly decreased to 67.96% CrI(020) and 81.15% CrI(110), respectively, after the degradation of the chitin by *O. arctica* YHY1. Further, peaks at $2\theta \cong 23$ related to the polysaccharide structure of chitin showed broad scattering due to the hydrolysis of the chitin, indicating a decrease in the crystallinity [7]. Likewise, a sharp peak detected at 31.46° was significantly reduced on the degradation of the chitin biomass.

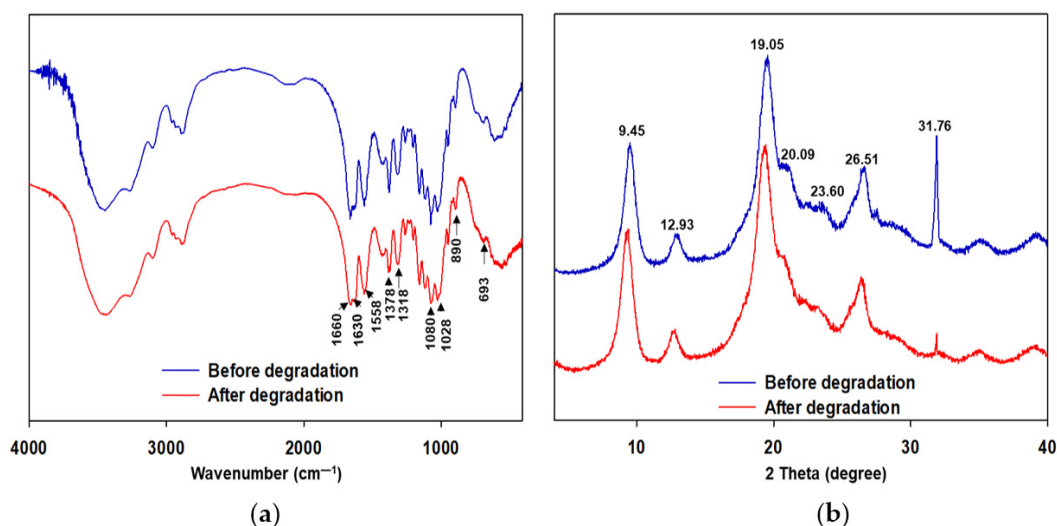


Figure 5. Structural changes in the chitin before and after degradation. (a) FTIR; (b) XRD.

4. Conclusions

The newly isolated halotolerant *O. arctica* YHY1 used in the present study generates a higher and stable electric current for a longer time by using seafood processing waste chitin as a carbon source. Monomeric GlcNAc and metabolites like acetate, lactate, and propionate produced during chitin degradation were consumed by strain YHY1 in a timely manner. The performance of the bacterium was improved by supplementing the MFCs with a salt concentration of 40 g L^{-1} . Further, *O. arctica* YHY1 can transfer electrons directly to the anode surface using membrane-bound cytochromes, with the partly involvement of extracellular redox mediators. Therefore, the findings of the present study can provide insights for utilizing chitin waste under high salt conditions, which can serve a dual purpose of recycling the seafood chitin biomass and generating electricity.

Author Contributions: Conceptualization, R.G.; Methodology, R.G.; Software, T.-R.C.; Validation, H.-J.K. and H.-J.L.; Formal analysis, J.-Y.C.; Investigation, R.G.; Resources, S.H.; Data curation, M.-J.S.; Writing—original draft preparation, R.G.; Writing—review and editing, S.K.B., S.-K.K. and D.-W.Y.; Visualization, S.-H.K.; Supervision, Y.-H.Y.; Project administration, Y.-H.Y.; Funding acquisition, Y.-H.Y. All authors have read and agreed to the published version of the manuscript.

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