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Isolation of nanocrystalline cellulose from tunicates

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ABSTRACT

Nanocrystalline cellulose (NCC) is a high value product, which consists of the nanoscale crystalline region of the cellulose polymer. Tunicates are marine invertebrate animals, comprised of tunic tissue, which acts as a skeletal structure, and internal organs. Tunic tissue is the only known animal source of NCC. Tunicates require a support to grow and spawn, which they find in the form of mussel socks in Prince Edward Island (PEI). This greatly affects mussel yields, and has become a nuisance to island fishermen. The present work is studying the feasibility of producing high value NCC from tunicates. Representing the first time tunicates have been utilized as a resource on PEI. NCC is isolated using prehydrolysis-kraft cooking-bleaching method. The yield of NCC from Vase and Club tunicate is 44 ± 8% and 32 ± 7% respectively. Club sourced NCC was determined to be 89 ± 7% crystalline as compared to 73 ± 6% from Vase. The average length of NCC was higher in the case of club (1567 ± 638 nm) than vase (1374 ± 600 nm), leading to aspect ratios of (90 ± 57) for club and (80 ± 60) for vase tunicates. The characterization of the obtained NCC by; powder X-ray diffraction (XRD), transmission electron microscopy (TEM), energy dispersive X-ray spectroscopy (EDX) and thermogravimetric analysis (TGA) are discussed in the paper.

1. Introduction

Aquaculture is a major industry in Atlantic Canada. According to Statistics Canada (2010), there has been 400% growth in aquaculture production since 1989. This industry now contributes roughly 50% of total aquaculture production in Canada, with the capacity to double production [1]. Over 70 different aquatic species are farmed in Atlantic Canada, the majority of which are salmon (85%), mussels (9%) and oysters (3%). Prince Edward Island is the largest producer of cultured mussels in Canada, contributing towards 80% of the total mussels harvested nationwide. The annual farm gate value of PEI mussels is $30 million, soaring to $75 million after processing [2,3]. With the remaining mussels produced in Nova Scotia, Newfoundland and Labrador, and Quebec. The mussel industry is however, being threatened by invasive species of tunicate, which are impacting the sustainability and productivity of local mussel industries. Tunicates are invertebrate filter feeding animals. They compete for food and space with other filter feeding animals like mussels, scallops and clams. Tunicates can also tolerate a wide range of water temperatures and salinity. Vase (Ciona intestinalis) and Club (Styela clava) tunicates are two of the most damaging invasive species, and are commonly found in PEI waters. They are very fast growing and can reproduce in 10 weeks, releasing more than 10,000 eggs [4]. Due to their high rate of reproduction, tunicates can quickly overgrow an area, replacing native species and becoming a major threat to biodiversity. As a preventive measure, larger mussel growers are trying to keep their mussels socks clean by either injecting high-pressure water or lime solutions. This cost farmers around 15% of their farm gate price annually, excluding the capital cost of the equipment [5]. The invasive tunicates however, could be used as a source for producing nanocrystalline cellulose (NCC). Systematic harvesting of tunicates for NCC production could potentially reduce the tunicate associated problems in farming mussels, while creating new economic opportunities moving towards bioeconomy.

Cellulose is well known as the most abundant organic polymer on Earth. There are many renewable sources of cellulose including plants [6–8], animals [9], algae [10], bacteria [11], and amoeba [12]. In the 1950s researchers began isolating nanocrystalline cellulose (NCC) from cellulose containing sources [6–10,13–15]. Interest in NCC has grown recently, as the demand for an alternative to nonrenewable fossil fuel based resources has increased [16]. NCC possesses many unique properties such as optical transparency [17], low thermal expansion [18], biodegradability, low toxicity [19] and low cost. These properties allow NCC to be utilized in a plethora of applications. Structural reinforcement of polymers is perhaps the most widely studied application [20]. However, NCC also finds use in barrier films [21], photonic crystals [22], shape-memory polymers [23], light-healable materials [24], drug-

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delivery [25], mechanically adaptive nanocomposites [26–28], biomedical devices [20,29,30] and membranes for water-treatment [31].

Tunicates tissue (also known as tunic tissue) is composed of cellulose microfibrils, which can be isolated to produce NCC. In fact, tunicates are the only known animal source of NCC [9,32]. Tunicates produce very high aspect ratio NCC (148 ± 147) as compared to NCC from biomass (23 ± 12) [18,33,34]. Tunicate cellulose is usually isolated as nearly pure cellulose Iβ crystals [32]. Due to high tunicate densities in some bodies of water, tunicate sourced NCC production at a large scale is possible [35]. PEI waters provide an abundant and accessible source of tunicate feedstock [5]. However, the objective of this paper is to investigate the isolation process and properties of NCC obtained from two species of tunicates, Vase and Club. The yield, properties and characteristics of NCC derived from Vase and Club are discussed in this paper.

2. Experimental

Club and Vase tunicates were collected from Malpeque Bay and Georgetown Bay, respectively. The tunicate samples were taken from several mussel socks, immediately placed into freezer bags and frozen. Frozen samples were thawed in a fume hood. The tunic was then liberated from the internal organs of the tunicate using a scalpel. The tunic samples were extensively washed with deionized water. Crystalline cellulose was then isolated from Club and Vase tunicates via the prehydrolysis-kraft cooking-bleaching method first reported by Koo et al. [36]. Fig. 1 explains the steps for isolation of NCC from tunicates.

The prehydrolysis, kraft cooking and bleaching steps were done in a PARR 4748 Large Capacity Acid Digestion Bomb equipped with a PTFE liner. Prehydrolysis and kraft cooking reactions were each carried out at 180 °C for 2 h. The bleaching step was carried out at 75 °C for a 1 h duration. A 20:1 ratio of solid to solution was used. The reactor was heated to temperature in a Paragon Sentry Xpress 4.0 oven, agitation was accomplished by physical shaking of the reactor at 10-minute intervals. Products were isolated via vacuum filtration using medium pore size fritted filters. The product was then dried in a vacuum oven at 60 °C for 24 h. The isolation and drying method was kept consistent for each step. The crystalline cellulose obtained following the bleaching step was centrifuged at 10,000 rpm and dialyzed against deionized water for 5 days to purify the product. Ultrasonication of the purified crystalline cellulose resulted in the formation of NCC.

2.1. Instrumentation

Powder X-ray diffraction (XRD) was performed to assess the percent crystallinity and average crystallite size of the isolated NCC. A Bruker AXS D8 Advance instrument was used. It was equipped with a graphite monochromator, variable divergence slit, variable antiscatter slit and a scintillation detector. Cu (Kα) radiation (λ = 1.524 Å) was used and the measurements were performed in air at room temperature from 2–60° (2θ). To assess the surface morphology transmission electron microscopy (TEM) micrographs were obtained on a JEOL 2011 STEM instrument. Elemental composition of the surface was investigated with the equipped EDX (Genesis) Energy Dispersive X-ray system. Dilute colloidal suspensions were cast onto pre-etched copper coated grids and air-dried prior to imaging. Thermogravimetric analysis (TGA) was utilized to provide thermal decomposition profiles for tunicate tissue and NCC isolated therefrom. Experiments were performed on a TA Instruments TGA Q500 under compressed air and nitrogen atmosphere up to 1000 °C, using a heating rate of 10 °C/min.

Table 1
Mass balance of the overall process.

Tunicate Species	Solid yield after Hydrolysis (%)	Solid yield after Kraft Cooking (%)	Solid yield after Bleaching (%)
Vase	67 ± 5	56 ± 4	44 ± 8
Club	57 ± 4	42 ± 3	32 ± 7

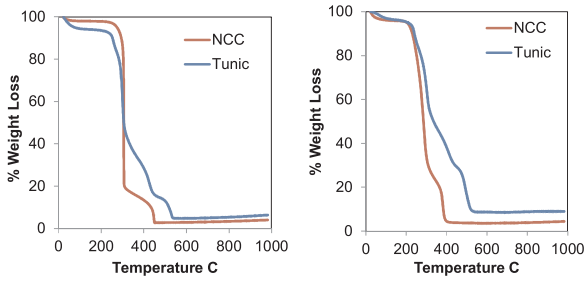

Fig. 2. TGA overlay of Club tunic powder and NCC prepared from the Club tunic powder (left), TGA overlay of Vase tunic powder and NCC prepared from the Vase tunic powder (right).

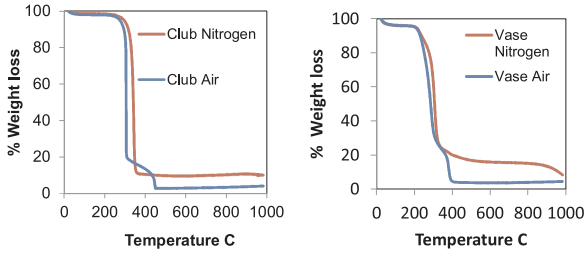

Fig. 3. TGA overlay of Club (Left) and Vase (Right) NCC in air and under nitrogen.

2.2. Materials

H_2SO_4, NaOH, Na_2S, NaClO, and acetone were purchased from Sigma Aldrich and used without further purification. Vase and Club tunicates were procured from Georgetown Bay and Mapleque Bay, PEI respectively (October 2016). The tunicate samples used in this work were removed from several mussel socks, placed in freezer bags and immediately frozen.

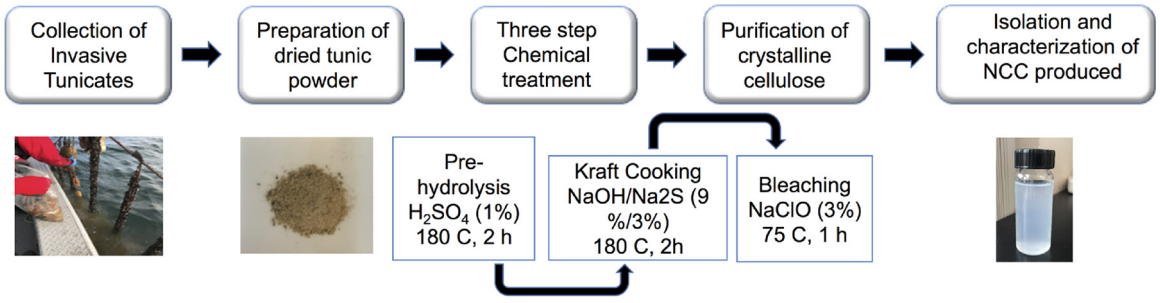

Fig. 1. Steps for producing NCC from tunicates.