Supporting Information

The Effect of Atomized Delivery of Nutrients on the Growth Characteristics and Microstructure Morphology of Bacterial Cellulose

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BC Pellicle Characterization Supporting Information

The following supporting information file is separated into five sections to outline the characterization that was performed on the BC pellicles grown using the SMND and ADND processes. The sections are:

(A) Equations to calculate growth characteristics of BC pellicles

(B) WAXS signals to calculate the crystallinity index (CI)

(C) Histograms and distributions of metrics measured from SEM Images

(D) Benchmarking of BC production to published intermittent nutrient delivery strategies

(E) Bacterial Cell Characterization using SEM Images

(A) Equations to Calculate Growth Characteristics of BC Pellicles

The following are the equations used to calculate relevant metrics to characterize the growth between static media nutrient delivery (SMND) and atomized droplet nutrient delivery (ADND). The variables for each equation are defined in the accompanying table for each equation.

Productivity (P)

$$P = \frac{m_{BC\ dry}}{V_{nutrient\ broth} * t_{incubation}}$$

m_{dry}	Mass of the dry pellicle (measured after lyophilization)
$V_{nutrient}$	Volume of nutrient broth supplied to culture (constant of 30 mL)
$t_{incubation}$	Total incubation time (constant of 90 h)

Carbon Conversion Efficiency (CCE)

$$CCE = \frac{m_{dry\ BC} * \%C_{BC}}{m_{Mannitol} * \%C_{Mannitol}} * 100\%$$

$m_{dry\ BC}$	Mass of the dry pellicle (measured after lyophilization)
$\%C_{BC}$	Percentage of carbon present per unit mass in BC pellicle
$m_{Mannitol}$	Mass of the Mannitol supplied to culture
$\%C_{Mannitol}$	Percentage of carbon present per unit mass in Mannitol

Density (ρ)

$$\rho = \frac{m_{BC\ dry}}{V_{BC\ dry}} = \frac{m_{dry}}{\frac{\pi}{4} \cdot D_{Petri}^2 \cdot t_{dry}}$$

$m_{dry\ BC}$	Mass of the dry pellicle (measured after lyophilization)
D_{Petri}	Inside diameter of Petri dish (constant of 100 mm)
$t_{dry\ BC}$	Thickness of the dry pellicle (measured after lyophilization)

Water Content (WC)

$$WC = \frac{m_{wet\ BC} - m_{dry\ BC}}{m_{dry\ BC}} * 100\%$$

$m_{wet\ BC}$	Mass of the wet/hydrated pellicle
$m_{dry\ BC}$	Mass of the dry pellicle (measured after lyophilization)

(B) Wide-angle X-ray Scattering Signals to Determine Percent Crystallinity

The signals produced from the Wide-angle X-ray Scattering (WAXS) equipment are shown in Fig. S1. These signals can be used to determine the crystallinity index (CI) of the BC samples, by applying the Segal Equation [Ref. 46]. The Segal equation has been applied to measure the crystallinity index of BC in previous literature [Ref. 20, 47, 48]. A total of three (3) replicates from both the ADND and SMND process were used to measure the crystallinity index.

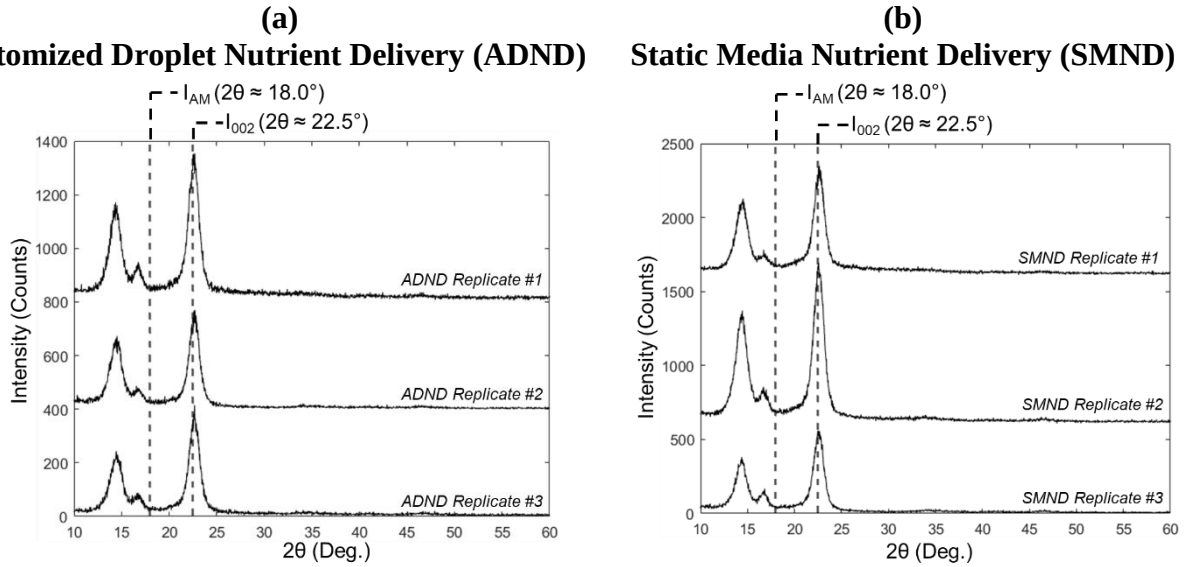


Fig. S1: WAXS signals for (a) ADND and (b) SMND BC samples

Segal Equation to Calculate the Crystallinity Index (CI)

$$CI = \frac{I_{002} - I_{AM}}{I_{002}} * 100\%$$

I_{002}	Intensity of the peak at 2θ ≈ 22.5°
I_{AM}	Intensity of the baseline at 2θ ≈ 18.0°

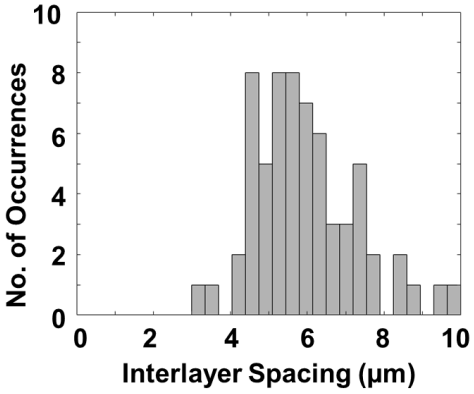
(C) Histograms and Distributions for Characteristics found from SEM Images

The histograms and distribution on the interlayer spacing, fibril diameter and pore diameter, and are presented in this section. These distributions were found through measurements taken through image analysis software.

Interlayer Spacing

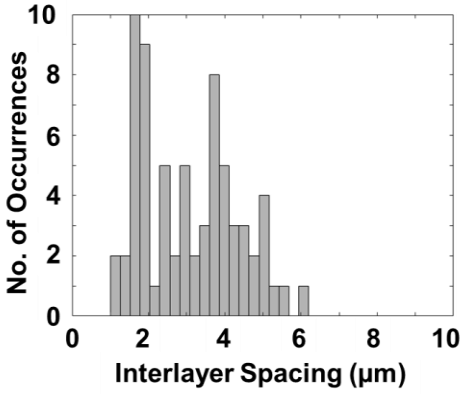
The interlayer spacing is the distance between adjacent dense fibril layers within the cross-section of the BC pellicle. The measurement was taken using ImageJ software [Ref. 44]. A total of seventy (70) measurements were taken from meso-scale SEM images (Fig. 3), to create the distributions shown in Fig. S2. The mean (μ) and standard deviation (σ) for these measurements are reported in Table 2.

(a) Atomized Droplet Nutrient Delivery (ADND)



μ = 6.22 μm
σ = 1.84
Coefficient of Variation (μ/σ)= 29.5%

(b) Static Media Nutrient Delivery (SMND)

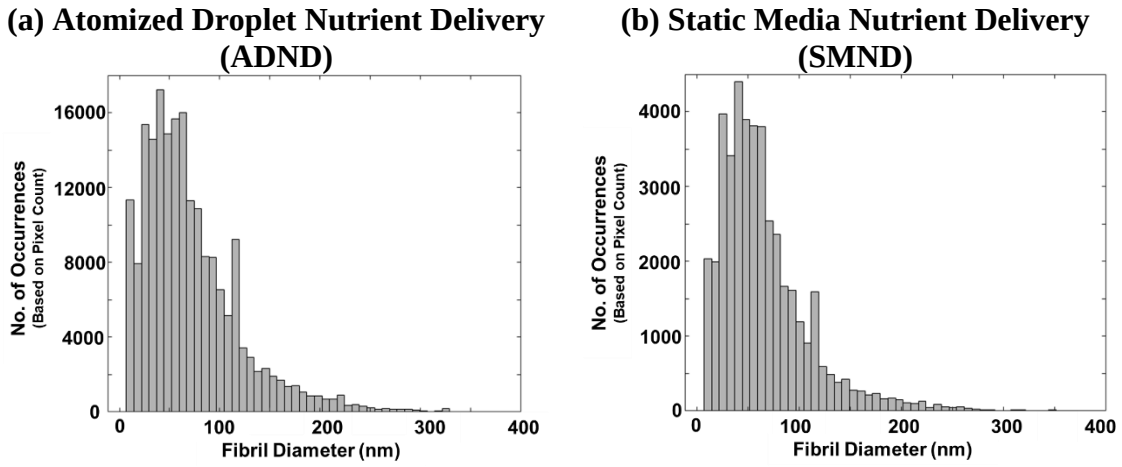


μ = 3.11 μm
σ = 1.26
Coefficient of Variation (μ/σ)= 40.5%

Fig. S2: Interlayer spacing distribution for (a) ADND and (b) SMND samples, measured from meso-scale SEM images (2.5-10 kX magnification)

Fibril Diameter

The fibril diameter was found using the DiameterJ plugin [Ref. 45] for ImageJ software [Ref. 44] DiameterJ calculated the fibril diameter based on the pixel count across the diameter of the fibril. The nano-scale SEM images (Fig. 4) were used to calculate the fibril diameter distribution, show in Fig. S3. The mean (μ) and standard deviation (σ) for these measurements are reported in Table 2.



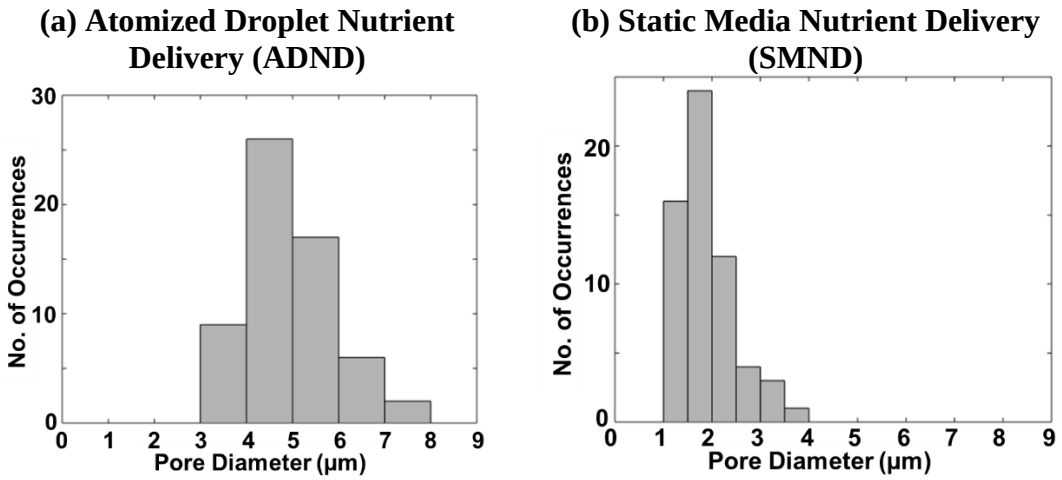
μ = 73.9 nm
σ = 15.4

μ = 67.7 nm
σ = 14.2

Fig. S3: Fibril diameter distribution for (a) ADND and (b) SMND samples, measured from nano-scale SEM images (100 kX magnification)

Pore Diameter

A total of sixty (60) measurements were taken from nano-scale SEM images (Fig. 4), to create the distributions shown in Fig. S4. The measurement was taken using ImageJ software [Ref. 44]. The mean (μ) and standard deviation (σ) of these measurements are reported in Table 2.



μ = 4.93 μm
σ = 0.98

μ = 1.87 μm
σ = 0.60

Fig. S4: Pore diameter distribution for (a) ADND and (b) SMND samples, measured from nano-scale SEM images (100 kX magnification)

(D) Benchmarking of BC Production to Published Intermittent Nutrient Delivery Strategies

Table S1 benchmarks the production results from this work (using the ADND process), to other comparable intermittent nutrient delivery processes reported in the literature. These processes are Hornung et al. [Ref. 41] and Hsieh et al. [Ref. 42].

Table S1: Benchmarking of relevant process parameters and metrics observed in intermittent nutrient delivery strategies present in literature

	ADND Process	**Hornung et al. [Ref. 41]**	**Hsieh et al. [Ref. 42]**
Measured Productivity ($g \cdot L^{-1} \cdot h^{-1}$)	0.73±0.04	0.16	0.28
Nutrient delivery method	Atomized micro-droplets	Aerosol micro-droplets	Bulk Fluid Delivery
Carbon source concentration ($g\ L^{-1}$)	40	20	25
Carbon source	Mannitol	Glucose	Mannitol
Droplet Size	>50 µm	<6 µm	Not reported
Feeding Frequency	12 h	5 min	24 h

(E) Bacterial Cell Characterization using SEM Images

A total of one hundred (100) measurements were taken from SEM images (Fig. S5(a)) at 50 kX magnification, to characterize the bacterial length (LB) of the *K. xylinus* cells used in the ADND and SMND process. The measurements were taken using ImageJ software [Ref. 44]. These measurements were used to create the distribution shown in Fig. S5(b). The mean (µ) and standard deviation (σ) of the bacterial length are 5.50 µm and 0,78, respectively.

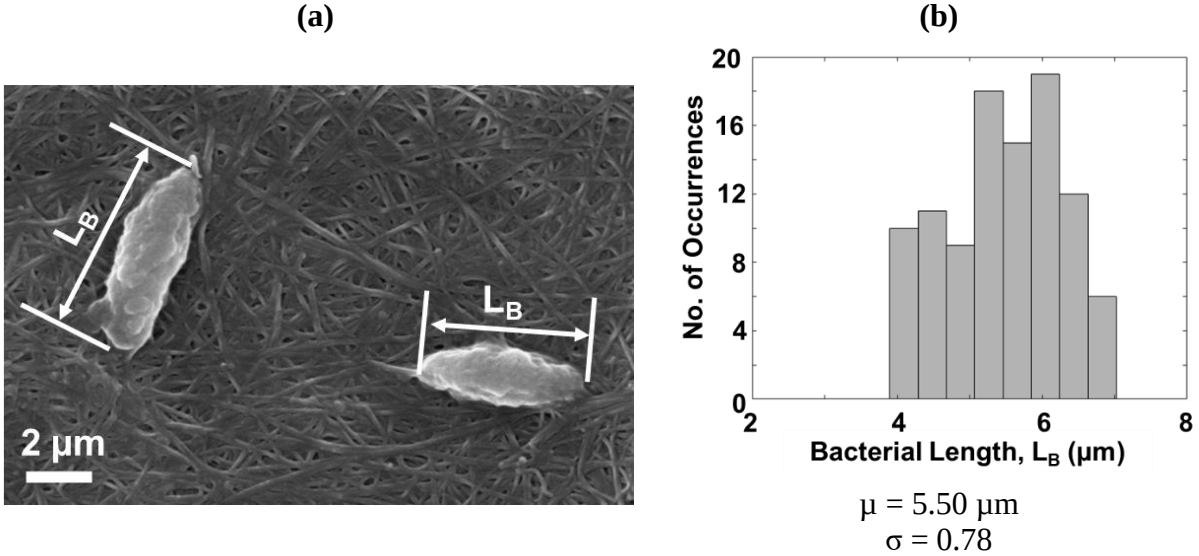


Fig. S5: Characterization of bacteria cells (*K. xylinus*) including (a) characteristic SEM image (50 kX magnification) of two bacteria cells within BC pellicle and (b) histogram of measured bacterial lengths (L_B)