

Shear stress quantification on a coupon surface in a CDC Biofilm reactor for varying rotational speeds

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ABSTRACT

Biofilms are communities of microorganisms, such as bacteria, yeast, and fungi, that attach to biotic or abiotic surfaces and work together to survive, mature, and spread. They remain a major concern in the food-processing industry, contributing to bacterial contamination and outbreaks. A bioreactor provides a controlled environment to grow biofilms under reproducible flow, nutrient, temperature, and shear-stress conditions for studying attachment, growth, and removal. A CDC bioreactor is particularly useful because it allows biofilms to grow on removable coupons under controlled dynamic shear conditions.

Past research suggests that shear stress can accelerate the attachment and development of biofilms. Experiments in CDC bioreactors established correlations between the rpm and biofilms growth and detachment processes. However, few studies have experimentally quantified shear stress levels in a bioreactor under operational conditions. Additionally, rotating flow in a bioreactor is complex and three-dimensional, which is a challenging environment for observation. To better understand true flow conditions an experiment was developed to varying the impellers rpm to observe the change in shear stress on the coupon substrates used to grow the biofilms.

We employed time-resolved Particle Image Velocimetry (PIV) to experimentally quantify the flow velocity field within the CDC Bioreactor. The particle motion and the estimated velocity fields depicts eddies and high mixing, high shear rates near wall and a periodic variation in flow features for different angular positions of the impeller near the coupon surface. This steeper velocity gradient in the boundary layer directly results in a higher shear stress, increasing the friction drag in the area of interest. For 100, 200 and 300 RPM impeller rotations, average wall shear stresses of 0.5946, 1.3752, and 1.8777 Pa were estimated. We aim to compare these findings with past research to improve our understanding of the correlation between an increase in shear stress and the development of biofilms.

1. Background

Biofilms are dense microscopic communities or microorganisms that attach to surfaces. A community is formed when the microorganisms produce and coat themselves in a mixture of sugars and proteins. This mixture acts as a protective shield against external forces, making them fairly resistant to typical chemical and heat related cleaning techniques. When resources decrease in a microorganism's biofilm colony, it detaches to form a new one. The detachment process and self-duplication makes it increasingly difficult to locate and fully remove biofilm colonies. Food processing environments provide the perfect breeding ground for these microorganisms (Olanbiwoninu & Popoola, 2023). Facilities with constant moisture, rich nutrients, and complex machinery surfaces allow biofilms to flourish. Surfaces, such as drains, pipes, conveyor belts, and other food contact surfaces, are most commonly at risk of biofilm growth and development (Simões et al., 2023).

Shear stress can directly impact the attachment and detachment process by adjusting the formation and structure of the collected microbial cells. This is important when removing biofilms, as insatiable bacterial structures tend to be easier to mechanically remove. Evaluation of shear stress can play a critical factor in understanding biofilm development, which can lead to a way of controlling these biofilm structures. Shear stress can additionally provide insight on how to slow down the microorganism colonies maturation in younger biofilms (Rochex et al., 2008). This could ultimately stop biofilm growth prior to the maturation of a microbe colony, eliminating microorganisms before they fully develop.

2. Introduction

Biofilms can be found and observed in many environments, including the food processing industry. Common foodborne illnesses, such as *listeria* and *salmonella*, come from the development of biofilms. In 2008, 80% of bacterial infections were due to biofilms being spread through the food processing industry. In total, approximately 48 million people have been infected by foodborne diseases caused by biofilms. This can cost upwards of 17.6 billion USD to compensate and treat those who contracted these major illnesses. Since biofilms proliferate, it is difficult to eliminate an entire community at a time. So even if food processing industries increase their cleaning and disinfecting process, there is still a high risk of recontamination.

Previous studies using CDC Bioreactors suggest correlation between shear stress and biofilms growth and detachment processes. Researchers have estimated shear stress in a CDC Biofilm reactor using Computational Fluid Dynamic (CFD) programs (Johnson et al., 2021). This demonstrated that shear stress, roughness and turbulence can influence biofilm development. These factors have been widely studied, but there is no clear indication of how fluid flow can impact biofilm growth in real-time.

CDC Bioreactors are commonly used in microbiology experimentation, providing initial insight and data of the flow within this controlled environment (Donlan & Costerton, 2002). We used Particle Image Velocimetry (PIV) to quantify velocity field information for flow within the CDC Bioreactor. PIV is a non-invasive optical method that uses recorded motion of flow tracer particles to determine spatially and temporally resolved velocity fields. When taking images, magnification and spatial resolution need to be established (Willert & Gharib, 1991). A higher magnification provides a near-wall high-resolution flow estimate used successively to analyze wall shear stress on coupons. The frame rate is also increased to limit particle shift in high-magnification imaging (Raffel et al., 2018). This was optimized so that the images will capture important details in the shear stress at the wall. Shear stress can be calculated using the following equation

$$\tau_{xy} = \mu \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right) \quad (1)$$

Where μ is the dynamic viscosity, $\frac{\partial u}{\partial y}$ is the change in the x-velocity along the y-axis, and $\frac{\partial v}{\partial x}$ is the change in the y-velocity along the x-axis.

Shear stress plays a crucial role in developing the structure of a biofilm's composition. In the food processing industry, biofilms naturally adapt in order to withstand varying shear forces that might be present. This means that if a biofilm often experiences a heavier shear stress, the bacteria can alter the attachment strength enhancing the adhesion of the biofilm. Biofilms become dense and thin with reduced microbial diversity as the microorganisms increase their metabolic activity due to the presence of shear stress (Rochex et al., 2008). Low shear stress allows for a thicker biofilm, with a loose attachment to any given surface (Prabhukhot et al., 2024). Previous work from our lab, in collaboration with the USDA, used a CDC Biofilm reactor to gather data on the growth rate of a multispecies biofilm consisting of *E. coli* and *R. insidiosa*. In this case, the data was gathered from coupons within a high shear flow condition (Prabhukhot et al., 2024). The calculations for quantifying high shear flow conditions experienced by the coupons, shown below, has been used to estimate shear stress on a CDC bioreactor coupon:

$$Re = \frac{\omega \alpha R_0^2 \rho}{\mu} \quad (2)$$

where ω = Rotation speed of baffle plate, α = Ratio of inner radius to the outer radius, R_0 = outer radius, ρ = density of fluid, μ = dynamic viscosity of fluid (Prabhukhot et al., 2024). The CDC Biofilm reactor has an outer cylinder radius of 3 cm and an inner radius of 2.25 cm. The transition Reynolds number for laminar flow to turbulent flow is calculated according to Bird, Stewart & Lightfoot (Bird, Stewart & Lightfoot. Transport Phenomena, 1960).

$$Re_{tran} = \frac{41.3}{(1-\alpha)^{1.5}} \quad (3)$$

The surface friction for turbulent flow is calculated using Blasius' formula shown below:

$$f = \frac{0.0791}{Re^{1/4}} \quad (4)$$

The wall shear stress is given by:

$$\tau_w = \frac{f \cdot \rho \cdot v^2}{2} \quad (5)$$

Though these equations are not used in this experimentation, it provides a theoretical benchmark for PIV testing in the CDC Biofilm reactor. These calculations identify the Reynolds number of each flow which will characterize the flow regime occurring at each RPM tested. When testing larger RPMs, there will be a transitional flow turning into a turbulent flow which can explain velocity fluctuations and wall shear stress patterns. These theoretical equations create a number-based analytical correlation for CDC Biofilm reactor studies, identifying a trend that can be used and compared to PIV-derived velocity and shear stress measurements. Physical experimentation will be able to solidify these predictions, with practices done in real-time (Prabhukhot et al., 2024).

3. Experimental set-up and procedure

The experimental setup and location of interest is shown in both **Figure 1** and **Figure 2**. The coupon material used is polypropylene, which is common within the food processing industry due to its high thermal resistance, cost-effectiveness and its FDA-approval for direct food contact.

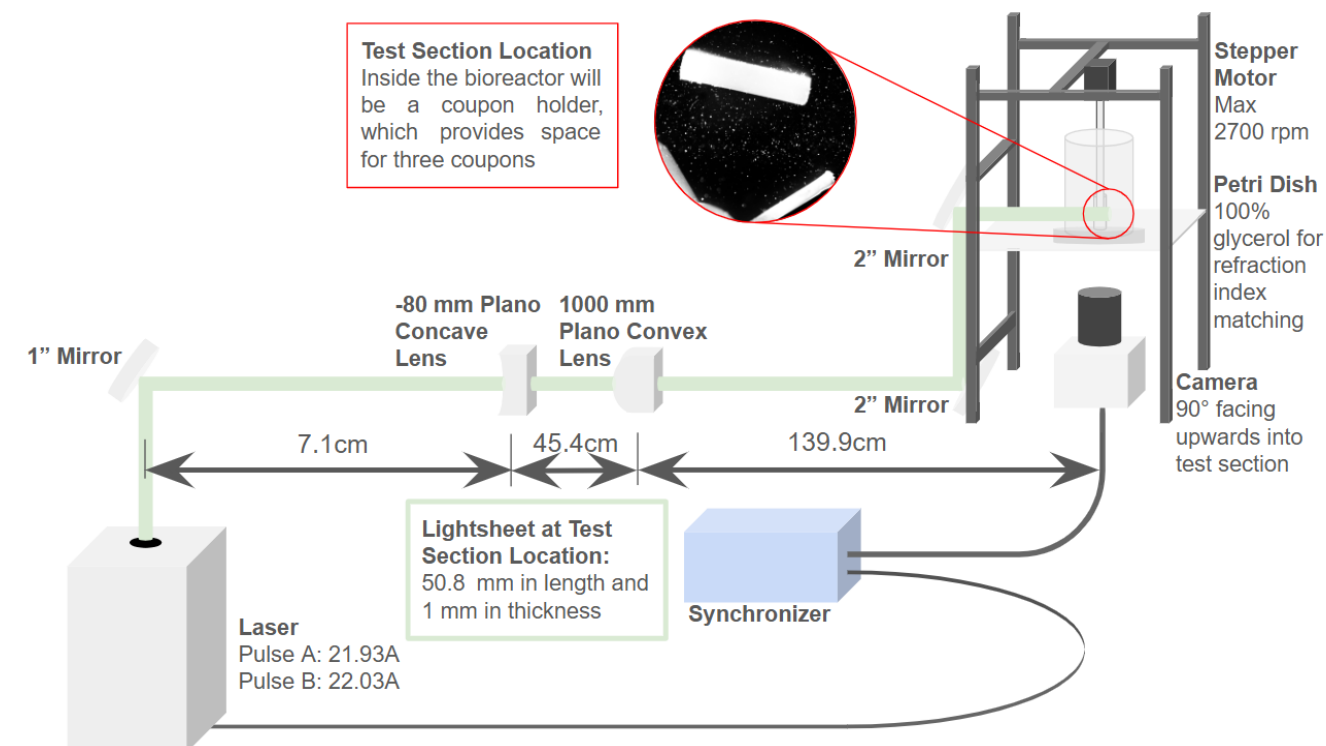


Figure 1. Experimental setup

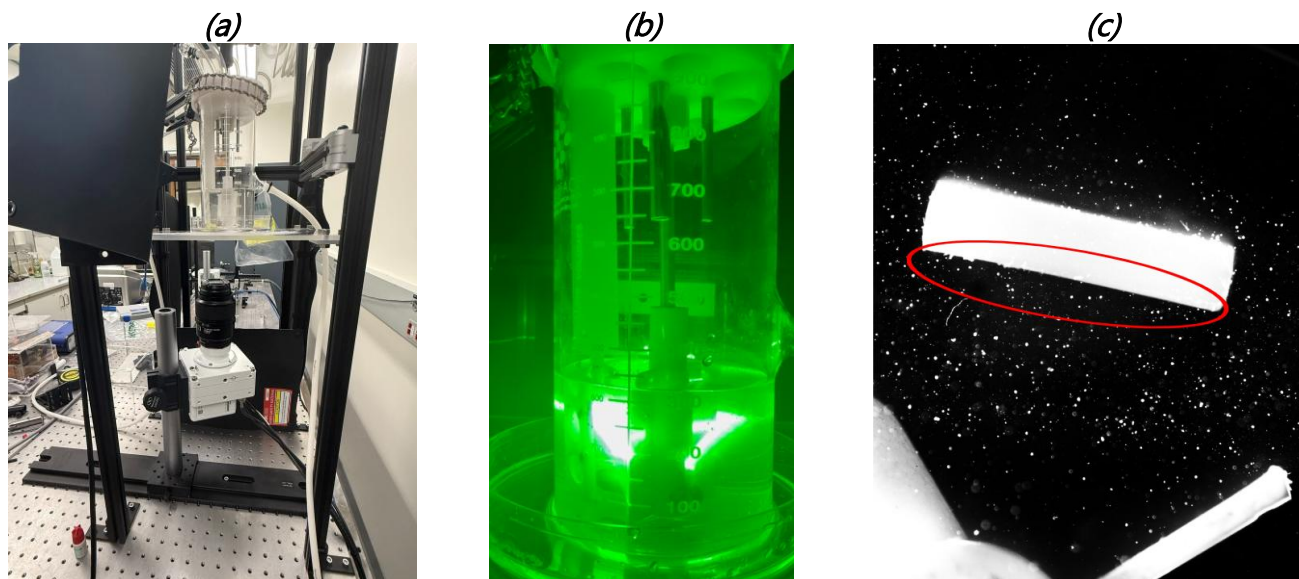


Figure 2. (a)Camera location in experimental setup (b)Coupon location and laser light sheet location (c)Location of interest within CDC Biofilm reactor

3.1 Hardware

The CDC Biofilm reactor was purchased in a package including the bioreactor itself and all adjacent parts, a variation of coupons, and a magnetic stir plate. The magnetic stir plate was designed to rotate the impeller once the bioreactor is placed on its surface. When designing the experimental

setup, we identified the ideal location of the camera, which should be orthogonal from the region of interest. This will prevent any geometric distortions when capturing the illuminated light sheet. Since a stir plate is designed to go in that location, an alternative rotation device must be used. An axle was designed to be inserted through the cap of the bioreactor and connected to the impeller below, as seen in **Figure 3**.

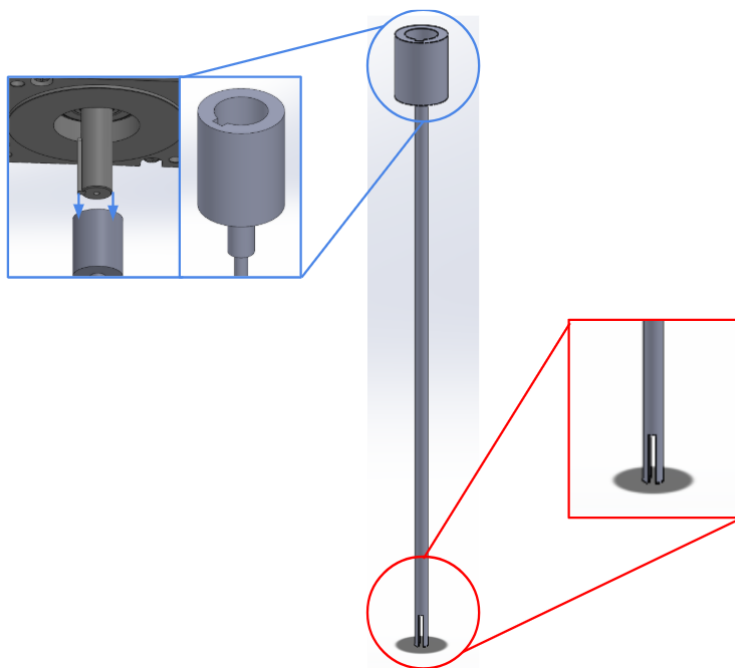


Figure 3: The axle insert modified to connect to stepper motor shaft and directly rotate the impeller, enabling imaging through the bottom of CDC bioreactor.

The axle consists of a mount that allows for it to be attached to a stepper motor, which was used in place of the stir plate. The bottom of the inserted axle contains a notch that clips onto the inside of the impeller assembly. The coupon has a diameter of 12.7 mm, and the impeller (or blade) has a tip-to-tip length of 56.1 mm. The stepper motor was then suspended above the bioreactor, aligning the area of interest with the laser light sheet. This allowed a clear view of the region of interest, while maintaining the ability to rotate the impeller at a specified RPM. Code was written to adjust the RPM, as needed, during experimentation. The RPMs being tested are 100, 200, and 300 RPM, which is consistent with the suggested operating range for the CDC Biofilm reactor. Through testing, it was observed that the bottom curved thick glass wall of the bioreactor created astigmatism effects leading to elongated particle images. This was resolved using a petri dish of *glycerol*, which has the same index of refraction as the Pyrex bioreactor. Prior to testing with particles, which can be seen in **Figure 4**.

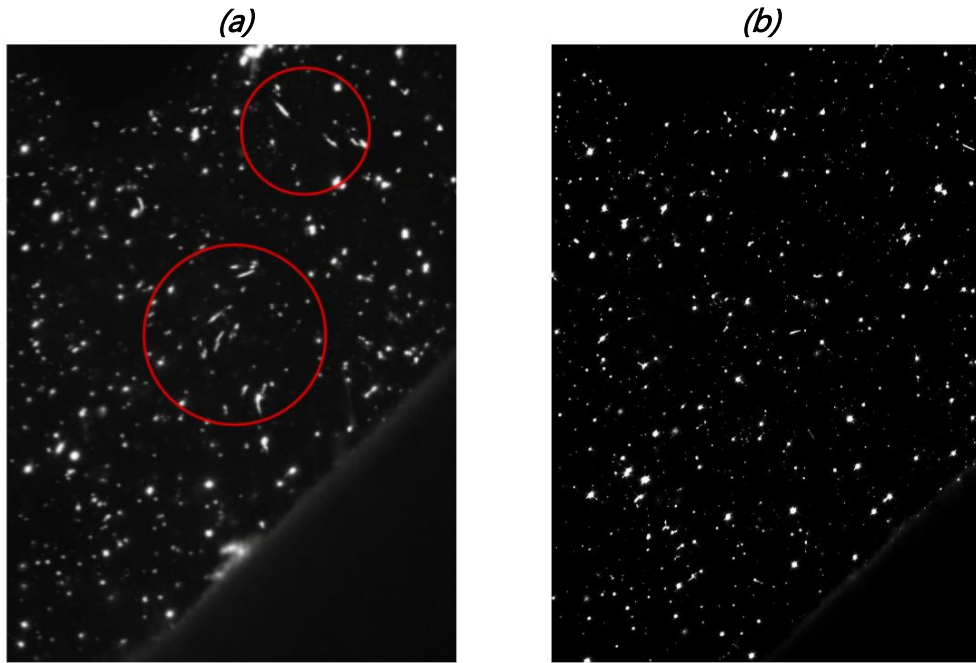


Figure 4. (a)Raw image without RI matching, distortions indicated by red circles, (b)Image with RI matching using glycerol bath.

3.2 PIV and Optical Arrangements

The PIV experimental setup included the Photonics Laser that will emit two laser pulses, one being at 21.93 Amps and the other being 22.03 Amps. The laser beam will then pass through two lenses, a -80 mm plano concave lens and a 1000 mm plano convex lens. The plano concave lens expands the length of the laser to 5 cm while the plano convex lens decreases the thickness of the light sheet to approximately 1mm, at the test section. The camera selected for use is the Phantom VEO E-340L high-speed camera. This camera was selected due to its high-resolution capabilities while maintaining a relatively high frame rate. The camera was set to capture images with a resolution of 1536x1536 pixels with an image rate of 1320 frames per second. The magnification was set to $9.77 \mu\text{m}/\text{pixel}$, which ensures velocity accuracy during observation. The camera exposure and laser pulses were synchronized using a LaVision PTU X synchronizer, and images were recorded using DaVis 11.0 software.

3.3 Vector Field Processing

The PIV velocity fields were processed using PRANA (<https://github.com/aether-lab/prana>), which is an open-source software. A static image mask was used to block any bright intensities from the coupon holder to prevent any unnecessary noise during the processing. Data is then extracted from each image, doing a cross correlation between image pairs. This helps identify the movement

of each particle throughout a certain period of time (Kähler et al., 2012). For this experimentation, we did a multi-pass deform processing, where the window size is 64,64 pixels for 100RPM, 128,128 pixels for 150 and 200RPM, and 256,256 pixels for 250 and 300 RPM. Window resolution and grid resolution are adjusted for additional passes. Each pass includes a minimum of 2 iterations and a maximum of 3 iterations. Validation using universal outlier detection and gaussian smoothing were applied for each deform iteration for convergence. (Scharnowski & Kähler, 2020; Westerweel & Scarano, 2005).

3.4 Post-processing

MATLAB codes were developed to post process the PRANA velocity fields to visualize velocity and estimate shear stress. The PRANA processed velocity fields were used to determine shear stress fields near the coupon surface. PRANA measures displacement in pixels/frame, which is converted to physical units of m/s using the image magnification and the frame rate. The velocity is shown in a quiver plot with an overlay of velocity contour, displaying the flow in real time. Shear stress is determined and calculated using *equation (1)*. The velocity gradients were calculated using MATLAB "gradient" command which uses central difference for numerical derivative estimation. The coupon wall coordinates are determined by detecting the changes in velocity magnitudes, changing from a finite value to 0 at the wall. The estimated wall coordinates were used to find the wall shear stress. The shear stress results were displayed using a contour plot, identifying the high shear locations near the coupon.

The wall shear stress fields are phase-averaged for two rotation cycles. Specific impeller angular positions are shown in **Figure 5** and **Figure 6**. The impeller is visible in the raw images only at two angular position, 0° and 180°, where the impeller blades are closest to the coupon. Based on the RPM and camera framerate, the exact number of frames ($2 * \Delta image$) for one full rotation is determined. The calculated ($\Delta image$) is also adjusted based on observation of specific frame numbers for 0° and 180° positions. The intermediate angular locations, i.e. 45°, 90°, 270°, and 315° were interpolated based on the $\Delta image$ range. Six angular locations are selected as reference to determine how wall shear stress changes as the impeller approaches and leaves the coupon. An example can be seen below:

$$image_2(180^\circ) - image_1(0^\circ) = \Delta image \quad (6)$$

$$Find\ 45^\circ \rightarrow \frac{\pi}{4} = \frac{\Delta image}{4}$$

$$image_1(0^\circ) + \frac{\Delta image}{4} = image_3(45^\circ) \quad (7)$$

This same calculation was done to find all other angles.

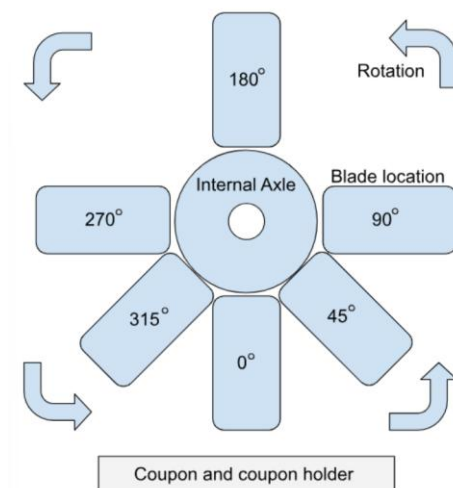


Figure 5. Impeller angular positions with respect to the coupon. Six reference angular positions are shown.

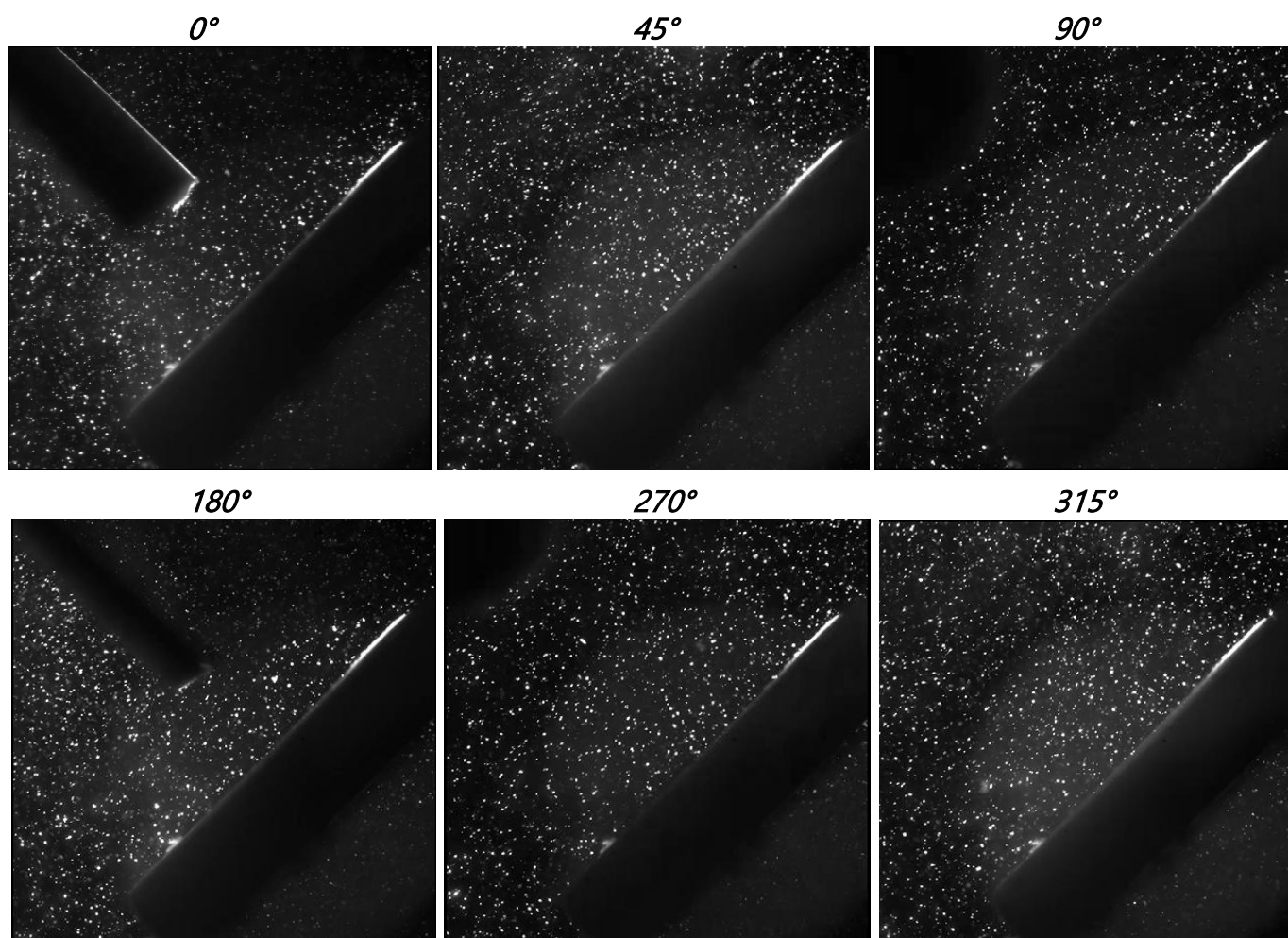


Figure 6. Raw PIV images for six reference impeller angular positions.

4. Results and Discussion

Time-resolved PIV measurements were successfully obtained within the CDC Biofilm reactor for 100, 200, and 300 RPM. Velocity vectors and corresponding magnitudes demonstrated a progressively increasing flow intensity as the bioreactors rotational speed increased. The increase in velocity magnitude with rotational speed was consistent with the expected hydrodynamic behavior of the stirred reactor system. At higher speed there was an increase in the angular velocity, influencing the fluid's circulation. Shear stress was correlated to the impeller periodicity, with a higher level of shear stress at the surface of the coupon during the highest speed in the flow, which was located at the impeller tip. Shear stress was found using *equation (1)*, where the velocity gradient and dynamic viscosity determined quantitative measurements.

4.1 Velocity and Shear Stress: 200 RPM

At 200 RPM, the flow field had a theoretical maximum velocity of 32.49 pixels/frame or 0.42 m/s, and an observed maximum of 47.36 pixels/frame. seen in **Figure 7**. The larger velocities in the observed experiment is likely due to minor noise and clustered particles appearing during the PIV experimentation. At 0° the flow accelerated occurs after the impeller's rotation, with the largest velocity occurring at the tip of the impeller. At 45° and 90° the velocity becomes more aligned along the coupon with minor rotational flows moving through the center of the flow. As the rotation continues, the acceleration zones appear to target the center of the coupon, with wake formation starting to appear more intense. This rotating flow moves down the coupons surface and eventually dissipates at the back end of the coupon. The velocity magnitude contours are normalized by the impeller tip speed ($U_{impeller}$), where $U_{impeller}$ is determined using the following

$$U_{impeller} = \pi \cdot D \cdot N \quad (8)$$

with D being the impeller distance from tip to tip, or 0.0561 m, and N is the rotational speed in $\frac{revolutions}{seconds}$. For 200 RPM the tip impeller speed is $0.5875 \frac{m}{s}$. In addition, each velocity figure skips every other vector to display an easy-to-follow flow visualization.

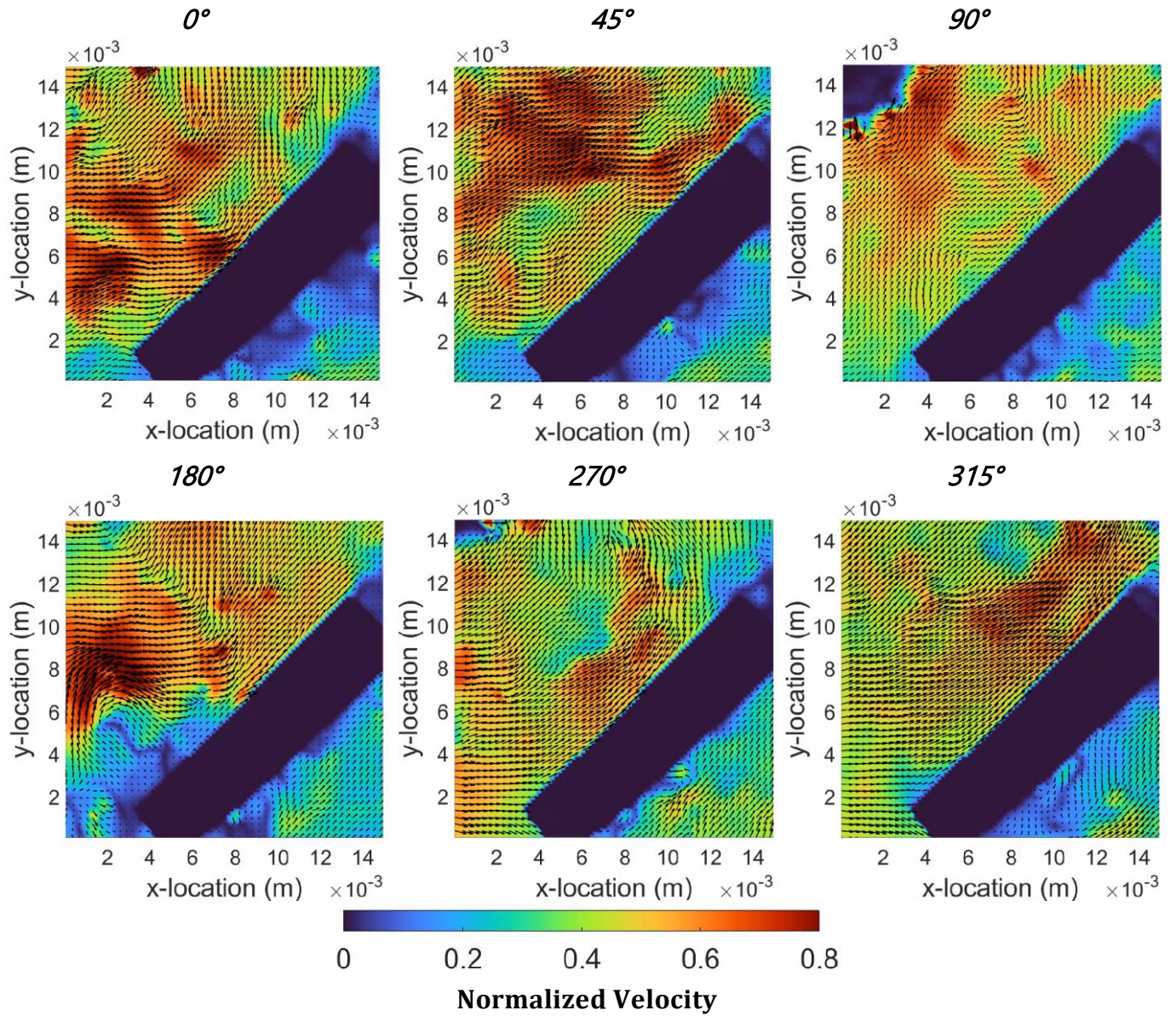


Figure 7. Velocity vectors overlaid on normalized velocity magnitude contour at 200 RPM for the six reference impeller angular positions.

The 200 RPM shear stress was determined and plotted using a contour plot, shown in **Figure 8**. The impellers rotation provides the largest shear stresses. At this RPM, there is a significant increase in shear stress along the coupon wall. The higher shear stress moves along the coupon wall following the rotation of the impeller. This is repeated at 0° and at 180° , identifying a pattern occurring at the start of each rotation. Enhanced vortex formation and flow separation are the reasons behind this increased shear stress location. The average shear stress throughout this rotation is 0.63 Pa. The average shear stress is determined by finding the mean shear stress over the grid of each image, then averaging over all of the image frames. The shear stress contours are normalized by the maximum shear stress in each frame, using the following equation

$$\tau_{w,norm} = \frac{\tau_w}{\tau_{w,max}} \quad (9)$$

where $\tau_{w,max} = \rho U_{tip}^2$ is determined using the following. For 200 RPM $\tau_{w,max}$ is 0.0105 Pa.

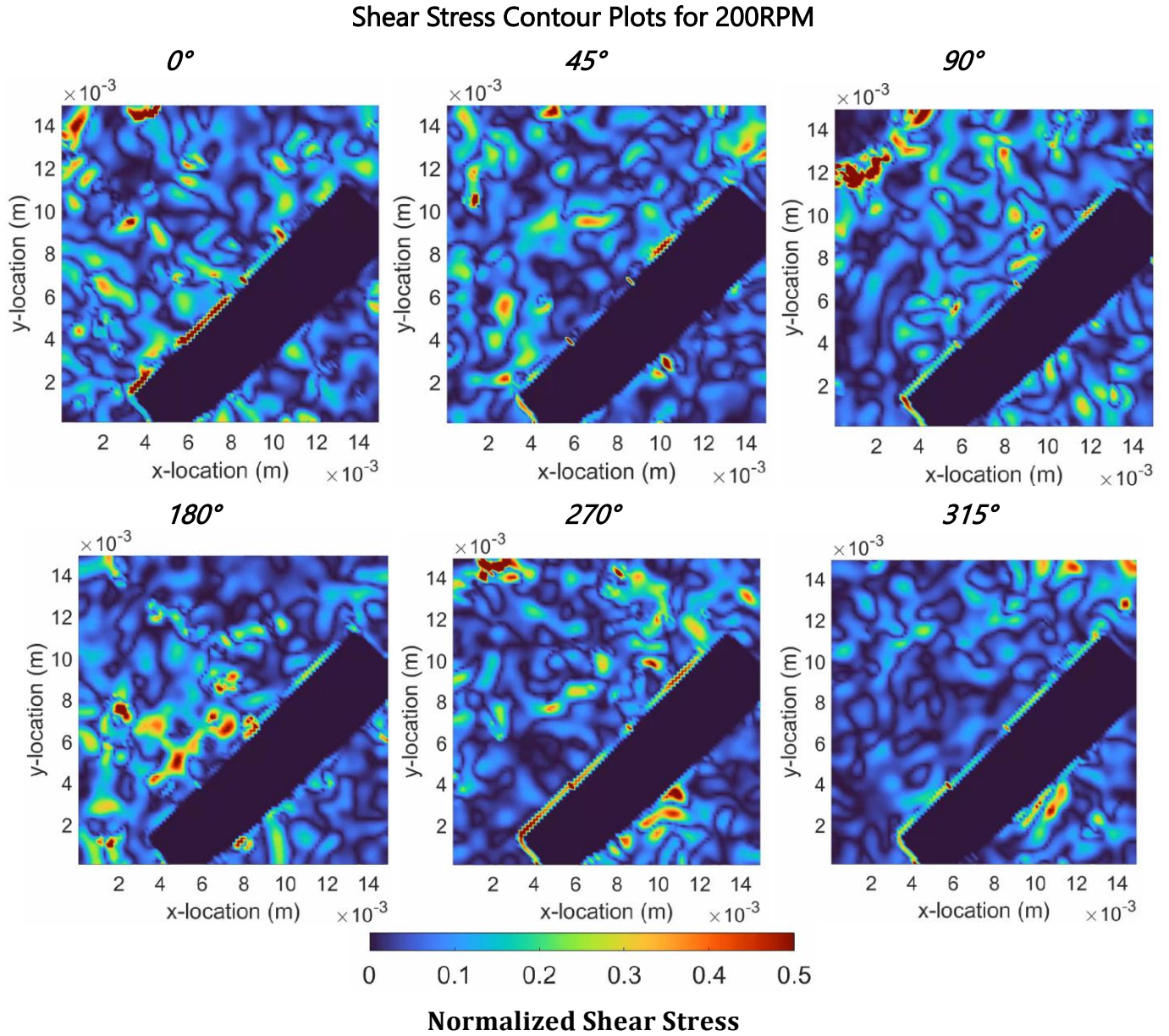


Figure 8. Shear stress contours at 200 RPM. Each subplot corresponds to the six referenced impeller angular positions.

4.2 Velocity and Shear Stress Overview

At 100 RPM, the shear stress is not distributed uniformly due to the coupon and coupon holders positioning. The highest shear stress occurs near the front of the coupon, as it is the first location that encounters the flow. The flow field had a maximum theoretical velocity of 16.28 pixels/frame or 0.21 m/s, and an observed maximum of 18.61 pixels/frame. The front to the middle of the

coupon experiences strong velocity gradients due to the boundary layer development and increase in flow acceleration as the velocity dissipates past the impeller as it rotates. There is a higher shear stress following the tip of the impeller, as the tip of the impeller should have the largest velocity during the rotation. Behind the coupon, the shear stress decreases as the flow separation causes a low-velocity wake. The only time this is not true is when a vortex develops, similar to what is seen at 315°. The average shear stress throughout this rotation is 0.26 Pa.

At 300 RPM, the flow field had a maximum theoretical velocity of 48.85 pixels/frame or 0.63 m/s, and an observed maximum of 59.57 pixels/frame. Once the impeller passes the area of interest, the velocity vectors are seen to point towards the coupons surface. It is more visible that the orientation of the coupon creates a blockage in the flow, forcing the fluid to accelerate around the coupon. Once the flow encounters the coupon, the flow rotates creating a vortex development and mixing. This phenomena can be seen clearly at 315°, where the flow is redirected producing substantial momentum transfer in a localized area. The start and end of the coupon experience high stress concentrations, indicating that there are vortex interactions at those locations, which can be seen in the velocity vector and contour images above. This is particularly evident for 45° and 315° images. Consistent with 100 and 200 RPM, the middle of the coupon holder experiences the largest shear stresses at the wall. It can be assumed that due to the increase in impeller speed, the shear stress and rotational flow hits the coupon surface later in the cycle. The average shear stress throughout this rotation is 1.0 Pa.

4.3 Wall Shear Stress Characterization for Each Cycle

The cycle wall shear stress was calculated using the shear stress and velocity flows for each RPM. This was displayed based on impeller angle and distance along the wall, which is shown in **Figure 9**. The wall shear stress calculation was determined by identifying the specific location that transitions from zero to non-zero numbers, using the masking images created for processing images. Once these points are identified, the first vector points are selected and used to set the wall boundary. The first point along the wall is 0mm, while the last point is 1.27cm, this distance was set as the coupon diameter is 1.27cm in length. The wall shear stress maps provide the full spatial and temporal distributions of wall shear stress over the coupon surface.

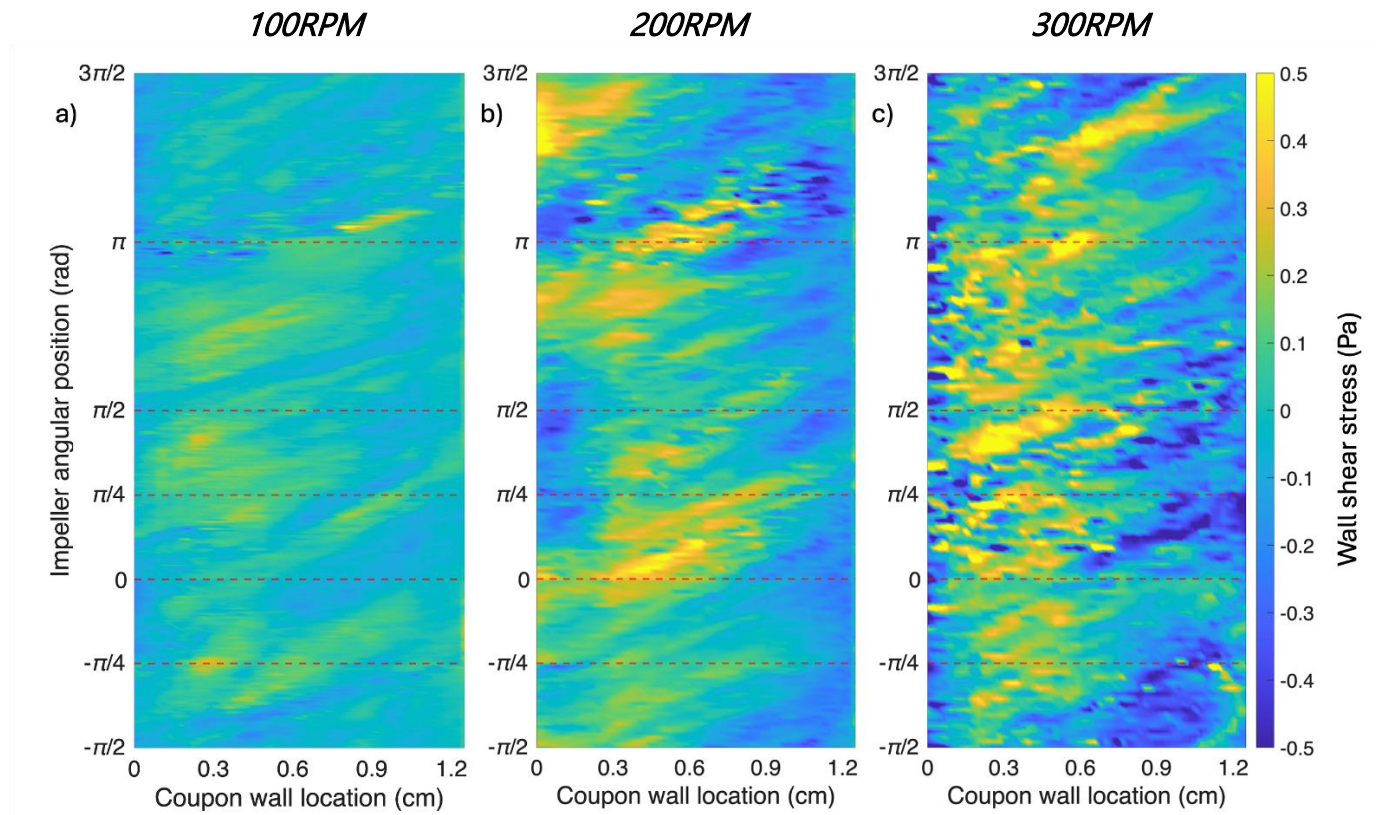


Figure 9. Instantaneous averaged wall shear stress distribution along the coupon wall for two cycles (complete impeller rotations $0^\circ - 720^\circ$) for 100, 200, and 300 RPM. The reference angular positions are marked using dotted horizontal lines.

Figure 10 and **Table 1** are condensed summaries of the information contained in **Figure 9**, which converts the time varying wall shear stress patterns into quantitative metric across all RPMs tested. The 100 RPM contour plot displays low wall shear stress values throughout two impeller cycles. The brightest regions occurs at 0.75 cm with another peak occurring closer to 0.3 cm, which correlates with the data observed in **Figure 10**. At 200 RPM, the wall shear stress significantly increases, in separate sections, with the highest peak being at 0.2 cm, which plateaus with minimal variation between 0.35 cm and 0.7 cm. The peak is 0.24 Pa, having a peak wall shear stress approximately $5/3$ the magnitude of the 100 RPM wall shear stress, which was 0.51 Pa. In **Figure 9**, at 300 RPM, the wall shear stress is at its highest, with the largest peaks occurring at 0.1 cm and 1.1 cm. In this region the peak wall shear stress appears slightly above 1.5 Pa with the average being 0.5 Pa. Throughout all RPMs, the shear stress is not uniformly distributed, but the data suggests that there is a presence of reoccurring flow structures. **Table 1** further extracts the single maximum values between all three RPMs.

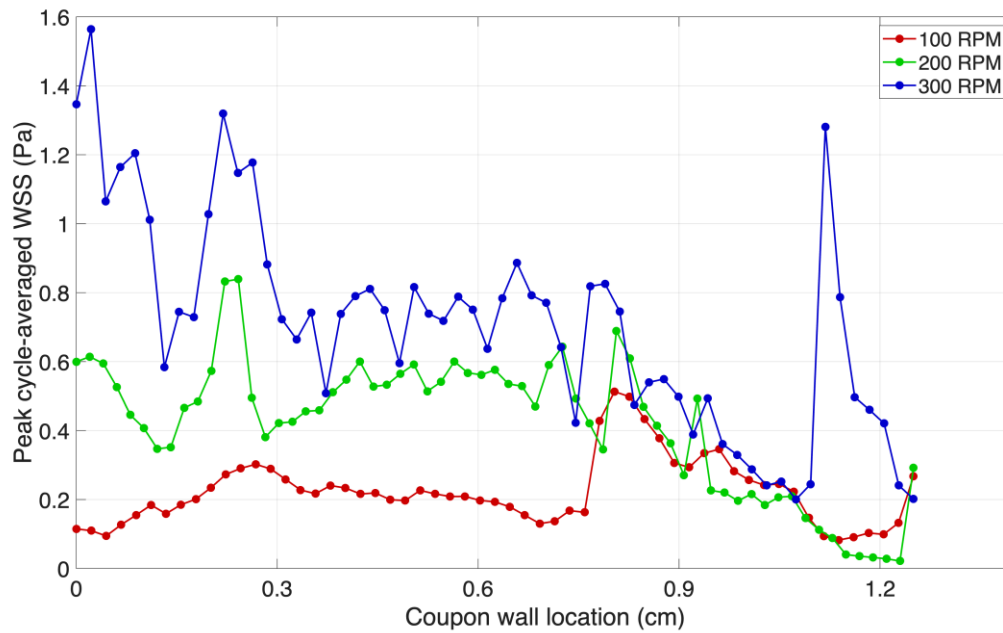


Figure 10. Change in shear stress at peak rotational cycle based on rpm

Table 1. Peak cycle average wall shear stress

RPM	Peak Cycle Average WSS (Pa)	Peak Coupon Wall Coordinates (cm)	Impeller Angle (π)
100 RPM	0.51	0.80	1.55π
200 RPM	0.84	0.24	1.62π
300RPM	1.56	0.02	1.42π

The peak locations indicate start of the impeller rotation cycles, with the highest wall shear stress locations occurring right before cycle times 0 and π . These cycle times coincide when the impeller is about to be at the closest location to the coupons surface during the rotation, shown in **Figure 11**. This figure displays the average shear changes during the impeller cycle. The rotation between positive and negative values indicates the coupon wall experiencing alternative flows during the cycle. 100 RPM displays small fluctuations in the mean cycle-averaged wall shear stress. It provides a smooth profile with stable flow fields, with a mean close to 0. As the speed of the impeller increases, it is noticeable that 200 RPM has larger oscillations, with more pronounced positive and negative values. The largest fluctuations occur at 300 RPM, with rapid changes in positive and negative shear, displaying unsteady flow conditions. As observed, the speed increase does not only increase the shear regions, but the temporal variability of the wall loading becomes more erratic.

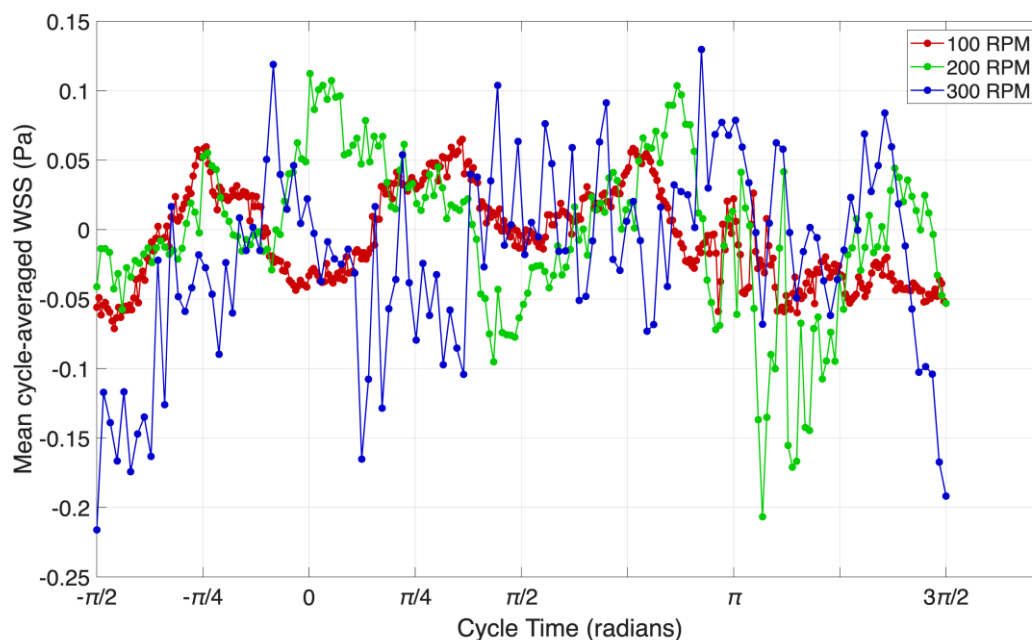


Figure 11. Average shear changes through impeller cycle

These results were compared to previous research that estimated the average shear stress in a CDC biofilm reactor, seen in **Table 2**. The shear stress predictions were calculated using *equation (2) to equation (4)*. The calculations were carried out for multiple materials, including stainless steel, which has a similar roughness to polypropylene. This allowed comparison with the current findings in Table 2. As seen, the average shear stresses are of the same order of magnitude, with the largest difference of approximately 23.4% being at 300 RPM.

Table 2. Transcendental calculation results vs. PIV analysis results

	Material	Reynolds Number	Approx. Roughness	RPM	Average Wall Shear Stress (N/m ²)
Predicted Eq. (2)-(4)	Stainless Steel (SS)	4072.0	$6.14 \cdot 10^{-5}$	100.00	0.37
		8143.0		200.00	1.21
		12215		300.00	2.45
PIV Analysis	Polypropylene (PP)	4072.0	$6.15 \cdot 10^{-5}$	100.00	0.59
		8143.0		200.00	1.38
		12215		300.00	1.88

4. Conclusions

PIV was used to measure fluid shear stresses on a coupon in a CDC Bioreactor operating at 100, 200 and 300 RPM. Images depict high mixing and vortex structures for different rotation rates of the impeller in a CDC bioreactor. Although the spatial distributions remain similar, the magnitude of the shear stress increased substantially with impeller speed as expected. The average wall shear stresses per cycle were of the same order of magnitude as those predicted by theory but differed by up to 23.4% at the highest shear rate. These results provide an understanding of hydrodynamic conditions within the CDC Biofilm reactor, which can be applied to our knowledge of biofilm formations.

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Nomenclature

τ_{xy}	Shear stress [Pa]
μ	Dynamic viscosity [Pas]
$\frac{\partial U}{\partial y}$	Change in x-velocity along the y-axis [1/s]
$\frac{\partial V}{\partial x}$	Change in y-velocity along the x-axis [1/s]
Re	Reynolds number
ω	Rotational speed of the baffle plate [rad/s]
α	Ratio of inner radius to the outer radius
R_0	Outer radius [m]
ρ	Density of fluid [kg/m ³]
f	Surface friction for turbulent flow [N]
$\dot{\gamma}_{xy}$	Strain rate [1/s]

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