Initial power measurements for a family of novel vertical-wheel bioreactors

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Abstract
The purpose of this investigation was to develop an initial set of Power number versus Reynolds number results for a family of vertical-wheel bioreactors. These bioreactors are increasingly being used for the manufacture of cells for cell therapy but have not been characterized according to this approach. A novel gravimetric method to measure power was used, and the validity of this method was assessed by measuring power for a standard stirred tank bioreactor with a Rushton impeller. The results of the gravimetric method were found to closely match those derived from traditional methods. The validated method was then used to measure the power draw and develop an initial set of Power number versus Reynolds number results for a family of vertical-wheel bioreactors.

KEYWORDS
Power number, Reynolds number, vertical-wheel bioreactor

1 | INTRODUCTION

For the manufacturing of cells used for cell therapy, traditional two-dimensional bench-scale methods to grow cells using flasks, roller bottles, or multi-layer plates are often insufficient to achieve clinically relevant numbers, especially for allogeneic applications.¹⁻³ Stirred bioreactors often need to be used, with the cells usually grown in suspension as single cells, as aggregates, or on microcarriers.¹⁻¹⁵

For most or nearly all applications of cell therapy, the scale required is usually far smaller than the 12 000–25 000-L stainless steel bioreactors often used for monoclonal antibody production. Single-use bioreactors (SUBs) offer sufficient capacity, up to 80–2000-L or more, as well as ease of use. Furthermore, SUBs for cell therapies can be custom designed for unique applications and need not adhere to the traditional design of a stirred tank with a horizontally rotating impeller.

One such set of custom-designed SUBs for cell therapy is the family of PBS Vertical-Wheel® (VW) bioreactors (PBS Biotech Inc., Camarillo, CA). Figure 1 illustrates the key design features of these bioreactors. These VW bioreactors are being used by numerous companies to successfully manufacture cells for clinical trials and have been the subject of many investigations, such as cell culture studies, computational fluid dynamic (CFD) modelling, and extracellular vesicle production.⁷⁻¹⁴,¹⁶⁻¹⁹ This publication focuses on the magnetic drive (MagDrive) series of PBS VW bioreactors. There are also pneumatic air-driven PBS VW bioreactors that have been used to manufacture cells, viruses, and other products.¹⁵,²⁰

There are many approaches to the characterization and scale-up of bioreactors.³⁻⁴,⁷⁻¹⁰,¹¹⁻³¹ To thoroughly characterize these VW bioreactors from an engineering
perspective, one needs to not only perform cell culture studies and CFD modelling but also characterize them in terms of their Power number versus Reynolds number relationship. The results can be used for many purposes, such as characterization of the flow regimes, scaling up at a constant volume-average energy dissipation rate per mass (VA-EDR or power per mass), and checking the accuracy of VA-EDR calculations from CFD studies. One can also potentially use the results to compare the performance of different bioreactor designs—such as VW bioreactors, traditional stirred tanks, wave bags, and orbitally shaken flasks—when operated at the same VA-EDR.

The purpose of this investigation was to measure the power draw and develop an initial set of Power number versus Reynolds number results for VW bioreactors.

2 MATERIALS AND METHODS

2.1 VW bioreactors

To try and cover a broad range of flow regimes from laminar to turbulent, many power measurements were performed with PBS 0.5-L VW bioreactors. A diagram of this vessel is shown in Figure 2. It has a relatively large VW impeller driven by external magnets and mounted on a horizontal axis. This VW impeller has a diameter of 7.26 cm. This represents 85.4% of the vessel diameter in the bottom U-portion. Under normal operation for cell culture purposes, this system consists of a vessel and a base drive unit placed inside an incubator. Akin to a spinner culture vessel on a magnetic stirrer, the magnets in the impeller are driven in a rotational motion by external magnets in the drive unit.

Power measurements were also taken for the PBS 3- and 15-L VW bioreactors. These are depicted in Figures 3 and 4. Under normal operation for cell culture purposes, these bioreactors are placed inside a housing unit with a bioreactor control system. Internal probes are used for the assessment and control of culture parameters, such as temperature, dissolved oxygen, and pH. The 3-L unit, like the 0.5-L unit, is a solid plastic vessel. The 15-L unit is a bag with a solid plastic impeller.

The PBS family of VW bioreactors is designed to be as geometrically similar as possible, within practical...
limitations. For the 0.5-, 3-, and 15-L vessels, the VW impeller diameter is 85.4%, 82.5%, and 82.6% of the vessel diameter in the bottom U-portion, respectively. This key design feature is maintained across scales. Less key features are more variable. For instance, as shown in Figures 2 through 4, the width of the VW impeller varies from 27.1% to 49.9% of the impeller diameter. Furthermore, under standard operating conditions, there are internal probes in the 3- and 15-L vessels but not in the 0.5-L vessels.

### 2.2 Equations for Power number and Reynolds number

When studies are performed regarding power input to stirred tanks, the results are typically presented using two dimensionless groups: Power number and Reynolds number.\(^{25-31}\) This classic approach was employed for this study, using standard Equations (1) and (2) as follows:

**Power number equation**

\[
N_p = \frac{P}{\rho n^3 D^5} \tag{1}
\]

where \(P = \text{power (from impeller into fluid; calculated here from experimental measurements)} \text{ (W)}, \ N_p = \text{Power number (dimensionless)}, \ \rho = \text{fluid density (kg/m}^3\text{)}, \ n = \text{agitator speed (rotations/s), and } D = \text{impeller diameter (m)}.

**Reynolds number equation**

\[
Re = \frac{\rho n D^2}{\mu} \tag{2}
\]

where \(\rho = \text{fluid density (kg/m}^3\text{)}, \ n = \text{agitator speed (rotations/s), } D = \text{impeller diameter (m)}, \ \mu = \text{fluid viscosity (Pa} \cdot \text{s)}, \ \text{and } Re = \text{Reynolds number (dimensionless)}.

For VW bioreactors, the impeller diameter is measured in the vertical dimension as the outermost diameter of each wheel, as shown in Figures 2 through 4. In each case, there are paddles that extend to this dimension. This is analogous to the paddles on a Rushton impeller that extend to the outside of the impeller diameter.\(^{26-30}\) In both cases, the impeller diameter is the swept diameter. The swept diameter is the appropriate length scale to use in these calculations.\(^{25}\)

### 2.3 Power measurement jigs

The target of this study was to measure power input across a range of scales from 0.5- to 15-L. Traditional measurements using a dynamometer or transmitting
torque metre were deemed impractical due to the low power levels involved for the PBS 0.5-L VW bioreactor. Others have noted these measurement challenges under low power scenarios.\textsuperscript{25,31} Furthermore, in this case, there is no vertical shaft running through the system to which traditional instrumentation is attached. Accordingly, a novel gravimetric method was used.

A measuring jig was built for use with the PBS 0.5-L VW bioreactor, as shown in Figure 5. It consists of a magnetic drive wheel mounted on an axle supported by ball bearings. A hollow spindle was machined and fitted over the shaft. A fine thread was wound in a thin layer on the spindle, and a small bag was attached to the loose end of the thread, into which a mass was placed. The apparatus was installed at either 2.2, 2.9, or 4.7 m above the ground. For measurements, a PBS 0.5-L VW vessel was mounted next to the magnetic drive wheel so that the drive magnets and the vessel impeller magnets were coupled. The falling mass turned the shaft, which then turned the drive magnets and the coupled impeller magnets. Special care was taken to prevent magnetic or electromagnetic induction effects from affecting the measurements by keeping the moving magnets away from any magnetic or paramagnetic materials. A Hall effect sensor connected to a digital tachometer was used to measure the rotation speed of the vertical wheel impeller. Similar jigs were built for use with the PBS 3- and 15-L VW vessels.

\section*{2.4 | Theory behind the gravimetric approach}

\subsection*{2.4.1 | Power measurements}

In this gravimetric approach, power was determined by calculating the energy transferred to the system by a
falling mass. The PBS VW bioreactor being tested was filled with a fixed volume of fluid and placed in the jig displayed in Figure 5. The magnets were allowed to couple. Known masses were placed in a small plastic bag and the bag was allowed to fall under the influence of gravity. During these measurements, the rotation of the impeller was driven strictly by the falling weight, with no connection to a normal motor drive. The total friction of the system (fluid drag + friction of the jig bearings + friction of the wheel bearings) soon arrested the acceleration of the mass. After an acceleration period of about 10 s, as presented in the results section, the mass fell at constant (terminal) velocity, with the system at a steady state. The air-flow resistance (drag) on the thin falling bag was deemed negligible, as the bag had a small horizontal cross-sectional area (<10 cm²) and a slow falling rate (<20 cm/s).

The measured parameters in this approach are the mass of the falling object (g) and the rotation rate (RPM) of the wheel at steady state, which represents the agitation rate of the vessel and is directly related to the linear velocity through the spindle diameter. The total power to drive the system is equal to the product of the velocity of the falling mass, the mass itself, and gravitational acceleration, as shown in Equation (3).

\[
P = \frac{E}{t} = \frac{F \times d}{t} = m \times g \times \frac{d}{t} = m \times g \times \frac{2\pi \times r \times \text{RPM}}{60} \tag{3}
\]

where \( P \) = power (W), \( F \) = force (N), \( E \) = energy (J), \( t \) = time (s), \( d \) = distance (m), \( m \) = mass (kg), \( g \) = acceleration due to gravity (m/s²), \( r \) = spindle radius (m), \( \text{RPM} \) = steady-state rotation rate of impeller (rotations/min).

2.4.2 | Friction measurements

Friction correction measurements were conducted to determine the fraction of the power that contributed to moving the VW and the fraction that contributed to overcoming the friction of the system.

For the friction measurements, VW vessels were placed in the jig empty to eliminate the opposition of the fluid drag force. The mass was then allowed to fall, as in the full VW vessel measurements. Because of the absence of the fluid drag force, any mass large enough to overcome the forces opposing gravity is continuously accelerated until it reaches the ground, yielding a non-constant RPM versus time graph. Through analysis of how the RPM versus time graphs change with different applied masses, this method yielded the minimum mass—and therefore torque—required to balance the friction torque in the system.

From Newton’s second law of motion for rotation, shown in Equation (4),\(^{[32]}\) the angular acceleration of the mass can be calculated.

\[
\tau = I \alpha \tag{4}
\]

where \( \tau \) = torque (N · m), \( \alpha \) = angular acceleration (rad/s²), and \( I \) = moment of inertia (kg · m²).

Newton’s second law of motion is expanded using the standard definition of torque: a value proportional to the force resolved in the direction perpendicular to the radius.\(^{[33]}\) Because all forces acting on this system are perpendicular to the radius, the entirety of the force being applied to the system is used in the definition of torque as shown in Equation (5).

\[
\tau = rF \sin \theta = rF \tag{5}
\]

where \( \tau \) = torque (N · m), \( r \) = radius (m), \( F \) = force (N), \( \theta \) = the angle between the applied force, and the radius of the spindle (rad).

In this system, torque is generated by three forces: the gravitational pull on the mass, friction, and air resistance. Assuming negligible air resistance to start, Equation (6) directly relates the force of friction in the system and the angular acceleration.

\[
\tau = I \alpha = rF(m - F_f) \tag{6}
\]

Rearranging this equation to solve for angular acceleration, one arrives at Equation (7).

\[
\alpha = \frac{gr}{I} m - \frac{F_f r}{I} \tag{7}
\]

where \( \tau \) = torque (N · m), \( r \) = radius (m), \( F_f \) = force of friction (N), \( I \) = moment of inertia (kg · m²), \( \alpha \) = angular acceleration (rad/s²), \( g \) = gravitational acceleration constant (m/s²), \( m \) = mass (kg).

If the maximum force of friction in the system, \( F_f \), is independent of mass, then a plot of angular acceleration, \( \alpha \), versus mass, \( m \), will be linear in accordance with Equation (7). If this is found, then the x-intercept, wherein the angular acceleration is zero, will yield the mass whose torque due to gravity is equal to the torque due to the friction of the system. In this case, any additional mass applied to the system will cause acceleration and the friction will remain at its maximum value. This mass is termed the friction correction mass, \( m_f \), as shown in Equation (8). Using this mass, the agitation power to the vessel can be calculated using Equation (9).
where $P = \text{power (W)}$, $r = \text{radius of spindle (m)}$, $g = \text{acceleration due to gravity (m/s}^2\text{)}$, $m = \text{mass of load (kg)}$, and $m_f = \text{friction correction mass (kg)}$.

### 2.5 Range of Reynolds number

Most experiments were conducted with water at $24^\circ\text{C}$, wherein the density and viscosity are approximately 997 kg/m$^3$ and 0.0009 Pa s, respectively.$^{34}$ Some were conducted at slightly lower or higher temperatures, with minor changes in density and viscosity. As explained in Section 3.1, when water was used, the friction correction appeared to be valid only for speeds above 26 and 35 RPM at the 0.5- and 3-L scales, respectively. Thus, using only water, this method was unable to determine power inputs at low rotation speeds and Reynolds numbers.

To characterize the bioreactors at lower rotation speeds and Reynolds numbers, the viscosity was increased using glycerol and various water–glycerol mixtures. By using pure glycerol, we were able to extend the measurements into the fully laminar regime, and thus determine whether our approach provided $N_p$ versus Re data with the expected slope in this range (–1 on a log–log graph).$^{26–29}$ The densities and viscosities of the glycerol and water–glycerol mixtures ranged from 1056 to 1265 kg/m$^3$ and 0.001 876 to 1.1786 Pa s, respectively.$^{35}$

### 2.6 Baffled stirred tank with Rushton impeller

To assess the validity of the novel gravimetric method, it was used to measure the power draw for a standard stirred tank bioreactor with a Rushton impeller. The results derived from our gravimetric method were compared to the published data derived from a traditional method for power measurement. A low friction, 90° pulley was added to the jig shown in Figure 5 to allow for measurements with a vertical impeller shaft.

This approach was originally based upon the assumption that there are well-accepted Power number versus Reynolds number relationships for Rushton impellers in baffled stirred tanks, as presented in decades-old original references $^{26,27}$ and subsequent textbooks.$^{28,29}$ Thus, a Power number of about 5–6 was expected in the turbulent regime. However, more recent updates to the Power number and Reynolds number relationship for this system have been made using more accurate power measurements and incorporating additional factors, such as disc thickness.$^{30}$ This updated analysis$^{30}$ was used to refine the expectations for the Power number.

The geometry of the six-bladed Rushton impeller and stirred tank used in this study is shown in Figure 6 using the terminology in Bujalski et al.$^{30}$ The dimensions (in mm) for the impeller and vessel are as follows: $D = 46.0$, $T = 138.0$, $x_1 = 1.86$, and $W = 11.97$. Using the first three of these dimensions, one can calculate the Power number, $N_p$, in the fully turbulent regime ($Re \geq 2 \times 10^4$), based on Equation (10) from Bujalski et al.$^{30}$:

$$N_p = 2.5 \left( \frac{x_1}{D} \right)^{-0.2} \left( \frac{T}{T_0} \right)^{0.065}$$

where $N_p =$ Power number (dimensionless) and $T_0 =$ reference tank diameter equal to 1 (m).

The calculated result is $N_p = 4.2$. This is for the standard geometry presented in Bujalski et al.$^{30}$ wherein $W/D = 0.2$. The impeller used in this study is wider, with $W/D = 0.26$. Based upon the correlations shown in Bates et al.$^{27}$ regarding the impact of blade width, $N_p$ should be higher by a factor of 1.3 for the wider impeller. Our experiments were done around a Reynolds number of 5800–6700, just below the cut-off of $2 \times 10^4$ for full turbulence.$^{30}$ Based upon the correlations shown in Bates et al.$^{27}$ this should result in a 0.3 unit drop in $N_p$. Thus, overall, our expected $N_p$ is 5.2, based upon the correlation of Bujalski et al.$^{30}$ with adjustments based upon the

**FIGURE 6** A baffled stirred tank with a Rushton impeller used to assess the validity of the gravimetric power measurement method
correlations of Bates et al.\textsuperscript{[27]} Furthermore, as expected in the near turbulent regime with a near-constant Power number, a plot of power per mass versus agitation rate should have a slope near 3 on a log–log graph.\textsuperscript{[29]}

3 \hspace{1em} RESULTS

3.1 \hspace{1em} Power measurements in VW bioreactors with water and/or glycerol

Multiple replicate power measurements were taken with PBS VW bioreactors containing water and/or glycerol, using the protocols described in Section 2.4.1. For each measurement, graphs of RPM versus time were produced. An example set of data is shown in Figure 7. There is an acceleration period of about 10 s, followed by a steady-state period of about 30 s, and ending with an arrested fall period. Each fall was arrested when the bag hit the floor, after which the momentum of the impeller would carry it through a few more, slower rotations. The data from the steady-state portion of the curve was used to determine the average steady-state RPM of the impeller and the terminal velocity of the falling mass. Each measurement was repeated 2–4 times. There was no difference in results between measurements taken across total drop heights of 2.2, 2.9, or 4.7 m. At all heights, a steady state was achieved in far less than 1 m and held constant until the weight hit the floor.

Average steady-state RPM values for replicates of the PBS 3-L VW bioreactor power measurements at 38 g are displayed in Table 1. One can see a typical coefficient of variation (CV) of about 1%. With water, for speeds of 26 RPM or lower in the PBS 0.5-L VW bioreactor, and 35 RPM or lower in the PBS 3-L VW bioreactor, the results were poorly reproducible, and/or a steady-state fall was only sporadically achieved. Thus, such data was not used to determine the Power number versus Reynolds number relationships. All data above this RPM range was used to determine the Power number versus Reynolds number relationships shown later in the results section. For this data, the range of CVs observed was 1–3%.

3.2 \hspace{1em} Friction measurements

Dozens of friction measurements were taken with empty PBS VW bioreactors, using the protocols described in Section 2.4.2. For each measurement, graphs of RPM versus time were produced. An example set of data is shown in Figure 8. After a start-up period of about 5 s where the mass was slow to move, there was an acceleration period, wherein the RPM increased linearly with time. In this case, the fall was arrested when the bag hit the floor,

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Mass (g) & Average of RPM at SS & Standard deviation of RPM at SS & Total average of RPM at SS & Total CV of RPM at SS (%) \\
\hline
38 & 97.89 & 1.68 & 96.43 & 1.08 \\
38 & 96.01 & 1.96 & & \\
38 & 95.38 & 1.77 & & \\
\hline
\end{tabular}
\caption{Replicate measurements of the terminal angular velocity of 38 g of mass from 3-L vertical-wheel vessel jig with 2.5 L water, 0% glycerol.}
\end{table}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Rotations_per_minute_RPM_versus_time_graph_for_power_measurement_of_PBS_3-L_vertical-wheel_vessel_2.5_L_water_0_percent_glycerol_38_g_mass}
\caption{Rotations per minute (RPM) versus time graph for power measurement of PBS 3-L vertical-wheel vessel, 2.5 L water, 0% glycerol; 38 g mass}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Rotations_per_minute_RPM_versus_time_graph_for_friction_correction_measurement_of_an_empty_PBS_3-L_vertical-wheel_vessel_15_g_mass_16.64_g_total_mass_with_bag}
\caption{Rotations per minute (RPM) versus time graph for friction correction measurement of an empty PBS 3-L vertical-wheel vessel, 15 g mass (16.64 g total mass with bag)}
\end{figure}
after which, the momentum of the impeller carried it through a few more, slower rotations. Acceleration was calculated from this graph by fitting the identified acceleration period to a linear equation and calculating the slope of the line. Figure 9 shows the identified period for the data in Figure 8 and the resulting best-fit linear equation. The angular acceleration of the 15 g mass (16.64 g when accounting for the mass of the bag)—represented by the slope of the line—is 4.36 RPM/s. This process was repeated for several masses, and the results are presented in Table 2. Each measurement was repeated 2–4 times. One can see a typical CV of about 6.5%, with some cases having lower values (about 1.5%). Across all the friction measurement data used to determine friction correction masses ($m_f$ values), the range of CVs observed was 1.5–13% and the range of linear-fit $R^2$ values was 0.981–0.999. In every measurement, the RPM versus time graph was linear. This indicates that air resistance on the falling bag of weights was negligible, as expected. If this were not the case, the resulting air drag would have led to a non-linear relationship with a declining slope. The friction correction mass was determined for each experimental vessel, as needed to account for friction heterogeneity between vessels of about 20%. For each vessel, not only for the PBS VW bioreactors but also for the baffled stirred tank with a Rushton impeller, the friction correction mass was determined and used to calculate the agitation power for the vessel according to Equation (9) in Section 2.4.2.

### Table 2

<table>
<thead>
<tr>
<th>Mass (g)</th>
<th>Average angular acceleration (RPM/s)</th>
<th>Average angular acceleration (rad/s²)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.64</td>
<td>1.24</td>
<td>7.80</td>
<td>-</td>
</tr>
<tr>
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<td>4.44</td>
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<td>22.64</td>
<td>9.92</td>
<td>62.36</td>
<td>1.57</td>
</tr>
</tbody>
</table>

3.3 Power number for a baffled stirred tank with a Rushton impeller

For the baffled stirred tank with a Rushton impeller, power measurements were performed in the Reynolds number range of 5800–6700, just below the cut-off of $2 \times 10^4$ for full turbulence. As mentioned in Section 2.6, the expected Power number in this range was 5.2. Our average measured Power number was 5.44, quite close to the expected value. The range of six measured values was 5.43–5.46, within 0.4% of the average, and showed a negligible trend with the Reynolds number ($R^2 = 0.9999$) with an exponent of 2.98. A log-log plot of power per mass versus agitation rate showed a strong correlation with an exponent of 2.98. This exponent was very close to the expected value of 3 in the fully turbulent regime. These results provide strong evidence that our gravimetric approach provides accurate power measurements for stirred vessels and is, therefore, a suitable method to use to measure the power of the PBS VW bioreactors.

3.4 Power number versus Reynolds number for PBS VW bioreactors

Figure 11 shows the Power number versus Reynolds number relationship for the PBS 0.5-L VW bioreactor, as measured via our gravimetric approach. The data are
separated by Reynolds number range. In the laminar flow regime, the expected slope on a log–log graph is \(-\frac{1}{3}\).\[26^{–29}\] This range is clearly seen for Reynolds numbers below 100, with a slope of \(-0.979\) and an \(R^2\) value of 0.9994 from a power-law fit. For the turbulent regime, the expected slope is zero.\[26^{–29}\] This range is clearly seen for Reynolds numbers above 7000, with a slope of only \(-3 \times 10^{-6}\) and an \(R^2\) value of only 0.112 from a linear fit. Between the laminar and turbulent regimes is the transitional regime, for Reynolds numbers between 100 and 7000.

Figure 12 shows the Power number versus Reynolds number data for the PBS 0.5-, 3-, and 15-L VW bioreactors. For comparison purposes, it also includes data from the Rushton studies (Section 3.3), as well as data from Bates et al.\[27\] for a pitched-bladed (PB) turbine in a baffled stirred tank. The data for the PB turbine was read off curve 6 in Figure 1 of Bates et al.\[27\] Findings from the data in Figure 12 are discussed in the following paragraphs.

Clearly, for the PBS 0.5-L VW bioreactor, and likely for the PBS 3-L VW bioreactor as well, the laminar regime extends out to a Reynolds number of about 100. This is a 10-fold higher range than that observed for the PB turbine data above and for many other impellers in baffled tanks, wherein the laminar regime typically extends out only to a Reynolds number of about 10.\[26^{–29}\] This difference may be because the PBS VW bioreactors do not have baffles, and as tested, did not have probes.

The data for the PBS 3-L VW bioreactor parallels the data for the PBS 0.5-L VW bioreactor. The Power numbers are slightly lower in the laminar regime but are similar to the PBS 0.5-L VW bioreactor’s numbers in the turbulent regime. Similar to the data for the 0.5-L VW bioreactor, the Power number data for the 3-L VW bioreactor shows no slope for Reynolds numbers of 7000 or higher. Thus, for both sizes of bioreactors, it appears that the turbulent regime is reached at a Reynolds number of 7000 or higher. In contrast, for the PB turbine in a baffled vessel, the turbulent regime is reached at a lower Reynolds number of about 1000, as one can see from the data from Bates et al.\[27\] For a Rushton turbine in a baffled vessel, full turbulence is not fully achieved until the Reynolds number reaches \(2 \times 10^4\) (i.e., 20 000) or higher.\[30\]

In the turbulent regime, the average Power numbers are 0.78, 0.81, and 1.11 for the PBS 0.5-, 3-, and 15-L VW bioreactors, respectively. These numbers are below the value for the PB turbine (about 1.4) and far less than the value for a Rushton impeller in a stirred tank (about 5). They are also below the values observed by Nienow et al.\[25\] for impellers in microscale bioreactors (about 1.5–2.3).
4 | DISCUSSION

An initial set of Power number versus Reynolds number results for PBS VW bioreactors has been successfully determined. The results can be used for many purposes, such as characterization of flow regimes, allowing for scale-up at constant VA-EDR, or power per mass, and/or checking on the accuracy of VA-EDR calculations from CFD studies. The results can also be used to compare power levels between bioreactors, such as the power inputs needed to achieve microcarrier suspension. If a CFD method can be validated against these experimental results, it can be efficiently used for the design and scale-up of bioreactors. With a validated computational approach, one can readily consider a variety of design options and scale them up at, for instance, constant VA-EDR values, while analyzing distributions to make sure no local maxima are hit that may lead to problems. This can circumvent the need to build proposed novel geometries for experimental characterization.

To obtain accurate Power number versus Reynolds number relationships for the PBS VW bioreactors, it was necessary to determine and correct for the impact of friction. Bujalski et al. discuss the impact of using a friction-free air bearing dynamometer versus the ball bearing type dynamometer used in previous studies. Simply put, if there is friction that is not identified and corrected for, the measured Power numbers will be higher than their true values. Aunins et al. corrected for friction by subtracting the torque levels measured for an impeller rotating in an empty vessel. They assumed, but did not test, that friction was independent of load. In this study, it was tested and found that friction was independent of load above a certain threshold load level (friction correction mass times gravity).

In our power measurements, friction was quite substantial. For instance, as described in Section 3.2, a friction mass correction of 11.41 g was determined for a typical 3-L vessel. When power measurements were taken with this vessel containing water and/or glycerol, friction consumed 7%–40% of the power drawn from the falling weight. Thus, it was critical to determine and correct for the impact of friction.

In the turbulent regime, the average Power numbers are 0.78, 0.81, and 1.11 for the PBS 0.5-, 3-, and 15-L VW bioreactors, respectively. The average of these averages (grand average) is 0.9. For the three different scales, the difference in each Power number versus this grand average is less than 24%. This is in substantial contrast to the results for stirred tanks with a Rushton impeller, wherein the Power number increases 2-fold when the width of the impeller blades is simply doubled. The key design features of PBS VW bioreactors have been maintained across scale-up from 0.5- to 15-L, resulting in only small differences in Power numbers.

The small differences in Power numbers may arise through certain differences in impeller geometry. The slightly higher Power number for the PBS 15-L versus the PBS 3-L may be due at least in part to the increase in the relative width of the impeller. The impeller width/diameter ratio is 0.27 for the 3-L versus 0.5 for the 15-L. The value of the Power number for the PBS 0.5-L VW bioreactor may be impacted by not only its impeller width/diameter ratio (0.35) but also its small number of blades (only four). The impact of impeller width/diameter ratio, as well as the number of blades and other geometric factors such as the presence of probes, will be part of future studies.

At PBS Biotech Inc., new designs and larger scales for VW bioreactors are in the works and, in some cases, already marketed. A second study of this type, covering not only the issues above but also new designs and scales, will soon be underway.

For the PBS 0.5-L VW bioreactor, the Power number decreases by only about 0.2 points, from 1 to 0.8, as the Reynolds number is increased from 1000 to 7000. In this near-turbulent regime, the average Power number is about 0.9. The PBS 0.5-L VW bioreactors are often operated in this near-turbulent regime, at Reynolds numbers of about 3000–6000. The larger scale PBS bioreactors are typically operated near or above a Reynolds number of 7000 or more, in the fully turbulent regime.

For instance, Dang et al. recommended target speeds for human induced pluripotent stem cell aggregate cultures to be 36, 26, and 17 rpm for the 0.5-, 3-, and 15-L PBS VW bioreactors, respectively. Using the Power numbers above, these speeds result in average power/mass levels of 0.0012–0.0013 W/kg. The corresponding Reynolds numbers are 4200, 11 000, and 19 800 for the 0.5-, 3-, and 15-L PBS VW bioreactors, respectively. These calculations are based upon density and viscosity levels for culture medium (DMEM) with 5% fetal calf serum.

Currently, PBS Biotech has tested agitation rates up to 100, 50, and 50 RPM for the PBS 0.5-L, 3-L, and 15-L VW bioreactors, respectively. These are the maximum tested agitation rates at which the magnets in the drive unit and in the wheel remain coupled. Using these rates, as well as the Power numbers, medium density, and medium viscosity cited above, one can calculate the current maximum average power/mass levels for each bioreactor that is currently rated. The resulting values are 0.024, 0.009, and 0.031 W/kg for the PBS 0.5-L, 3-L, and 15-L VW bioreactors, respectively.

Published cell harvesting protocols are being developed to use mechanical stress in addition to enzymatic dissociation for microcarrier culture. One such protocol published by Nienow et al. involves the exposure of
microcarrier cultures to brief (~7 min) periods of high agitation, sufficient to provide local maximum power per mass levels of roughly 0.11 W/kg. While the current maximum average power per mass values for the PBS VW bioreactors are 3.5–12-fold lower than 0.11 W/kg, Nienow et al. state that local maxima values range from 10 to 100 times the average. Thus, the power per mass values achieved at the maximum rated agitation speeds for PBS VW bioreactors may well be suitable for this cell harvesting protocol. As development continues, higher agitation rates will be tested to determine true maximums. Speed levels may be further increased, if needed, by using larger magnets.

5 | CONCLUSIONS

An initial set of Power number versus Reynolds number results for PBS VW bioreactors has been successfully determined. A new gravimetric approach to measure power was validated. This approach will be used for additional studies.

AUTHOR CONTRIBUTIONS
Matthew S. Croughan: Data curation; formal analysis; investigation; methodology; supervision; validation; visualization; writing – original draft; writing – review and editing. Daniel Giroux: Conceptualization; data curation; formal analysis; investigation; methodology; visualization; writing – original draft. Omokhowa M. Agbojo: Data curation; formal analysis; investigation; methodology; visualization; writing – review and editing. Erica McCain: Data curation; formal analysis. Nathan Starkweather: Data curation; formal analysis. Samantha Guerra: Data curation; formal analysis. Yas Hashimura: Conceptualization; investigation. Brian Lee: Funding acquisition; project administration; resources; supervision. Sunghoon Jung: Supervision; writing – review and editing.

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CONFLICT OF INTEREST
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