Effect of simulated microgravity & hypergravity on growth characteristics in cyanobacteria *Synechocystis* PCC 6803

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Contents

1 Introduction and background **4**
1.1 Gravitational force 4
1.2 Weightlessness and Microgravity 5
1.2.1 Simulated microgravity 6
1.2.1.1 Clinostat 7
1.2.1.2 Other devices for microgravity simulation 7
1.3 Hypergravity 8
1.3.1 Centrifuge 8
1.4 Cyanobacteria 9
1.4.1 *Synechocystis* PCC 6803 11

2 Objectives **12**

3 Objective A: Dynamics of cell under clinorotation **13**
3.1 Dominant forces acting on a cell under clinorotation 13
3.1.1 Gravitational force 13
3.1.2 Centrifugal force 13
3.1.3 Viscous force 14
3.2 Parameters describing motion of a cell during clinorotation 15
3.2.1 Velocity of sedimentation 15
3.2.2 Radius of circular trajectory 15
3.2.3 Centrifugal displacement 16
3.2.4 Minimizing centrifugal force 17
3.3 Case study: Parameters describing the motion of cyanobacterial cell during clinorotation 18
3.4 Inference obtained from above discussion 19
3.5 Experiment: Calculating sedimentation velocity 21
3.5.1 Aim 21
3.5.2 Purpose of experiment 21
3.5.3 Objectives 21
3.5.4 Apparatus 21
3.5.5 Mathematical relations 22
3.5.6 Method or procedure 22
3.5.7 Observations 23
3.5.8 Results 23

4 Objective B: Growth characteristics of *Synechocystis* under altered gravity **24**

4.1 Growth characteristics 24
4.1.1 Growth curve 24
4.1.2 Cell viability 25
4.1.3 Cell morphology 25
4.2 Studies under simulated microgravity and hypergravity 26

5 Discussion and conclusion 27

Bibliography 29

1 Introduction and background

1.1 Gravitational force

Gravitation or the gravitational force, in simple terms, is an attractive force that exists between two objects having certain masses. This force inversely varies with the distance that separates the two masses. When more than one objects are present, the total force on any one of the objects is the vector sum of forces on it due to all the other objects. Mathematically, this force is expressed by Newton's equation for gravity.

$$\boxed{F = \frac{Gm_1m_2}{r^2}} \tag{1}$$

where, $G = 6.667 \times 10^{-11}\ Nm^2kg^{-2}$ is called the Universal Gravitational constant, m_1 and m_2 are masses of objects 1 and 2 respectively and r is the distance separating the two objects. The direction of this force is along the line joining the two masses.

The fact that this gravitational force is inversely proportional to the square of distance between the two objects has some important consequences. Firstly, as the objects move further apart from each other, the force that was trying to bring them closer decreases. Secondly, even though this force reduces in magnitude with distance, it never really vanishes, unless the objects are separated by an infinite distance. This implies that the range of this force is infinity. So, no matter where an object is in the universe it will experience a non-zero gravitational force.

Gravity on Earth is characterized by a constant acceleration, expressed by the symbol g. The corresponding force of gravity vector points towards the center of Earth. Mathematically, it is defined as follows,

$$\boxed{g = \frac{GM_{Earth}}{R^2_{Earth}}} \tag{2}$$

where, M_{Earth} and R_{Earth} denote the mass and the radius of Earth respectively. The value of g is 9.8 ms^{-2}.

This definition allows us to simplify (1) to the following form,

$$F = mg \tag{3}$$

Equation (3) defines the force that an object placed on the surface of Earth would experience. Note that, the symbol g is strictly used in context of Earth's gravity.

1.2 Weightlessness and Microgravity

Weightlessness is the absence of weight. When an object is placed on the surface of Earth, the downward force that it experiences (see equation (3)) is balanced by another force acting upwards (due to Newton's second law), This that object remains stationary and doesn't accelerate further into the ground. This normal force is that object's weight. Under variation of normal force, the object will experience either increased or decreased weight. Normal force arises from contact forces such as friction. These are forces that act on an object when it is in contact with other objects or any surface.

Weightlessness isn't the absence of gravitational force, rather it's the state where gravitational force is balanced by another equal and opposite force. *True* zero-gravity, on the other hand, is a state when an object doesn't experience any gravitational force. But gravitational force can only be absolutely equal to zero at infinity (i.e. when distance between two masses is zero). This is because gravitational force has an infinite range and disappears only at infinity.

Let's illustrate weightlessness with a simple example. Imagine you are standing inside an elevator (see 1).

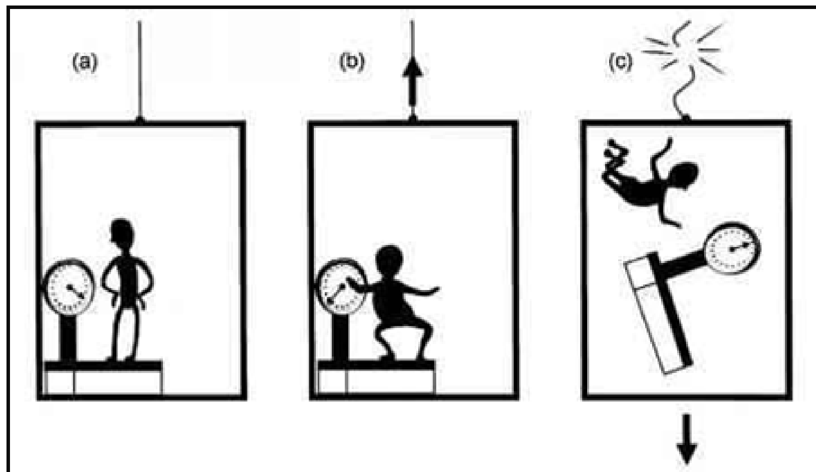

Figure 1: Weight and weightlessness. Image source: Fossil hunters

In the first case (from left), the elevator is at rest. Here, your weight (normal force, N) will be,

$$N = mg \tag{4}$$

When the elevator is moving upwards, your weight will be the gravitational force and the force (ma). This extra term comes into existence because of the fact that the elevator is an accelerated frame of reference. Mathematically,

$$\boxed{N = mg + ma = m(g + a)} \tag{5}$$

Above equation tells us that when the elevator is moving upwards, you will "feel" an increased weight.

When the elevator is moving downwards, your weight will be the gravitational force minus the force (ma). This extra term, as mentioned earlier, comes into the picture because the elevator is an accelerated frame of reference. Mathematically,

$$\boxed{N = mg - ma = m(g - a)} \tag{6}$$

Above equation states a condition of near-weightlessness or microgravity.

Finally, if the cable of the elevator is cut, you and the object will fall under the same acceleration, g. This means $a = g$ in equation (6). This yields,

$$\boxed{N = 0}$$

and this state is weightlessness. It is evident from the fact that your weight will be zero in this case.

A person in space is supposed to be weightless. In practice though, we observe microgravity or near-weightlessness condition in space. This can be due to various reasons such as uneven mass distribution in the spaceship, vibrations due to thrusters or other modules, etc. Typical value gravitational forces experienced by astronauts in space are in the range 10^{-3}g to 10^{-6}g.

1.2.1 Simulated microgravity

Microgravity provides a low stress environment. Different organisms will behave differently in such environments. The problem is microgravity is not achievable for prolonged periods on Earth. But we can try to mimic the effects produced in micro-gravitational conditions and a condition thus obtained is called simulated microgravity (SMG).

SMG is achieved through instruments like clinostats, RPMs (Random Positioning Machines), diamagnetic levitation apparatus, Rotating-wall vessels (RWVs) among others.

Drop towers and parabolic flights produce micro-gravitational conditions for very short duration. These cannot be employed for studies which involve monitoring growth of an organism.

1.2.1.1 Clinostat A clinostat (see figure 2) is a device that rotates the biological sample about a single axis, which is orthogonal to the g-vector. Its construction, generally, consists of a horizontally placed disk or cylindrical sample holder which is rotated with the help of a motor placed behind it. The disk/cylinder rotates in a horizontal plane powered by an electric motor. Rotation speed and other parameters are controlled externally by an electronic controlling device. The basic principle behind working of clinostat is time-averaging the g-vector to a near zero value by continuously changing the direction of g-vector, in the frame of reference of the sample.[1]

Figure 2: 2D clinostat. Image source: Researchgate scientific image library

What regulates clinorotations is the angular or rotational speed. Lower speeds will cause the cells to settle by the wall of vessel (or sediment). While at higher speeds sample will experience non-negligible external forces. That's why, selection of an optimal rotation speed is essential. And this speed depends on factors like the sample being used, its suspension media, etc.

A cell inside a clinostat will revolve in small circles because of the forces acting on it. These forces are discussed in detail in latter sections. In brief, these forces prevent the cell from sedimentation which prevents the cell from experiencing any normal force and thus no weight.

1.2.1.2 Other devices for microgravity simulation A 3D clinostat is a device which takes rotates the sample about two independent axes, by changing its orientation continuously, while maintaining constant rotation speeds and directions. If the speeds and directions vary, then the device is called a Random Positioning Machine (RPM). Unlike clinostat, RPM's trajectory can be randomized by constantly changing

its direction. RPMs are usually better suited for complex organisms like plants.

Other devices (and methods) used to simulate microgravity are rotating wall vessels (which has functioning just like a clinostat except constant nutrition is supplied to the cells inside it), diamagnetic levitation (where magnetic forces are used to counter the effect of gravity), parabolic flights (where a plane follows a defined parabolic curve to achieve microgravity for as short as 20s) and drop towers (where an object is dropped from large heights in a low pressure environment to simulate free fall).

1.3 Hypergravity

Hypergravity is the condition when the gravitational accelerations exceed the value of 1g. It can range from a few g's to several hundred thousand g's [4]. Hypergravity can be easily achieved through the use of centrifuge and ultra-centrifuge machines.

Due to inertial mass, objects have a tendency to travel in a straight line. When observed in inertial or non-accelerating frames, they do travel in a straight line. But when their frame of reference is a non-inertial frame like rotating frames, they experience an outward force which appears to distort this path.[5] These are pseudoforces. Pseudoforces are fictitious forces which act on an object in an accelerating frame of reference.

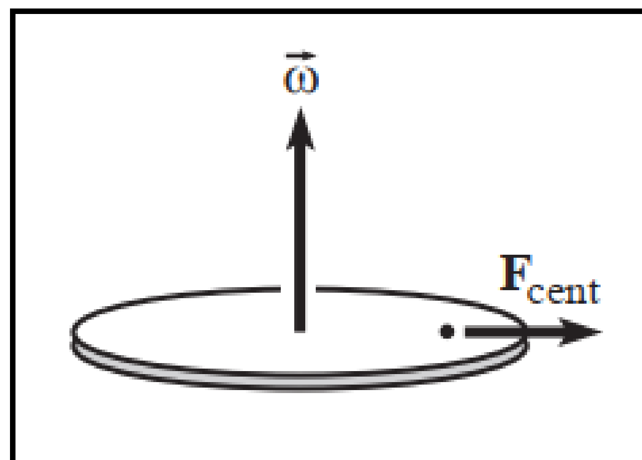

Figure 3: Example of a carousel as a non-inertial frame. Image Source: Morin (2007) [10]

1.3.1 Centrifuge

A centrifuge (see figure 4) is a device which consists of a powerful electric motor at center. Attached to the axis of rotation, there are slots to place small tubes which contain samples. To prevent heating due to high-speed motor, rotor chamber is evacuated

and isolated. Also, balance of weight of the sample inside the centrifuge is important.

A typical centrifuge can perform a few thousand rotations per minute. An ultracentrifuge are an improvement over normal centrifuge since they can perform more than 70,000 rotations per minute or even more.

To study how a centrifuge works we can look at the example of a carousel or a merry-go-round. A person on this carousel will *feel* a force which will push that person further from the center (see figure 3). This force is a pseudoforce called centrifugal force. And this is the force which is responsible for increased weight inside a centrifuge.

Figure 4: Centrifuge. Image source: Labconsult

1.4 Cyanobacteria

Cyanobacteria is a class of prokayotes which are one of the oldest organisms on Earth. They perform oxygenic photosynthesis in a process very similar to that of plants. Some species in this class fix atmospheric nitrogen. These bacteria have numerous applications such as for bio-fuel production, medicine, food supplements, etc [11].

These bacteria are known to survive in environments such as freshwater, oceans, soil, and bare rock. But they can also survive extreme hot and dry conditions, and are being studied to include in future space explorations [6]. Optimum temperature for fast growth for cyanobacteria is in the range $30 - 40^{o}$C but can vary depending upon the species, environmental conditions (including illumination) and nutrient levels [7].

Cyanobacteria are found in various forms for instance filaments, spirals, individual spherical cells or even in colonies. In general, their size varies from 1 to 2 microns or a few microns.

Cyanobacteria contain two distinct reaction centers: P700 (photosystem I) and P680 (photosystem II). Each of these reaction centers is associated with a light harvesting antenna - phycobilisome. These absorb light in 500-720 nm range. Chlorophyll a is a commonly found photosynthetic pigment in these reaction centers. Chlorophyll b and c are other photosynthetic pigments that are sometimes found in cyanobacteria.

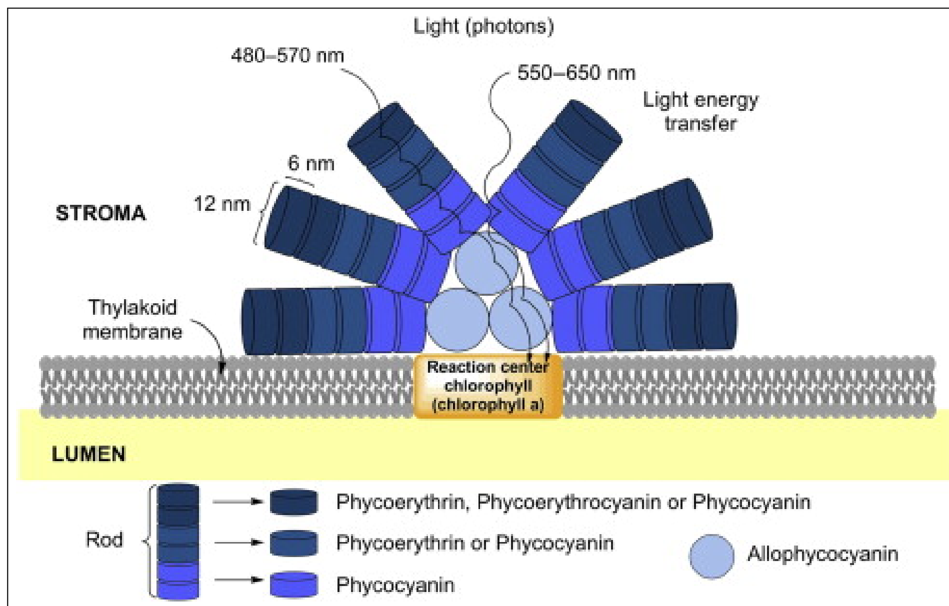

Figure 5: Typical phycobilisome structure. Image source: Dumay et. al (2014) [9]

Phycobilisomes (see figure 5) are protein complex composed of a core substructure and peripheral rods. The core is made up of allophycocyanin to which rods made of stacked disks of phycobiliproteins (mainly phycocyanin, but sometimes phycoerythrin or phycoerythrocyanin are also present) are attached. Phycobilisomes absorbs 30-50% of the incident light and passes the energy to the reaction centers, thus, acting as light harvesting antenna.[8]

1.4.1 *Synechocystis* PCC 6803

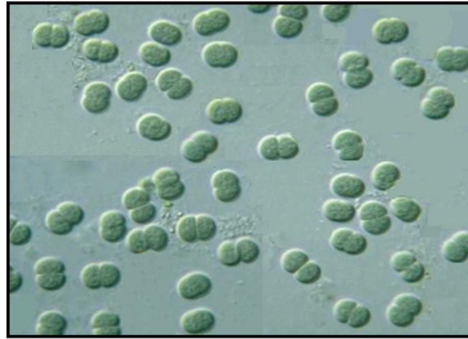

Figure 6: *Synechocystis* PCC 6803. Image source: Alchetron

Synechocystis (species: S. sp. PCC 6803) (see figure 5) is a unicellular cyanobacteria. Like other cyanobacteria it can perform photosynthesis. It grows autotrophically (in the presence of light) as well as heterotrophically (in the absence of light). They are spherical in shape and green in colour (due to photosynthetic pigments). It is found usually in freshwater sources and its photosynthetic machinery is similar to that of plants.

Why *Synechocystis*? *Synechocystis* is a model organism which means it has been extensively studied and its whole genome has been sequenced. Hence, provided we observe functional changes during a study, we can trace these changes back to a particular gene.

The growth and nutritional requirements of this particular cyanobacteria are simple. Also, doubling time of *Synechocystis* is lesser. Additionally, since its photosynthetic machinery is similar to that of plants, these are preferred for photosynthetic study too.

2 Objectives

- **To determine the dominant forces inside a 2D clinostat and to study their effects on bacterial cells.**
- **To study the effect of simulated microgravity (SMG) and hypergravity on growth curve, cell viability and cell morphology in *Synechocystis* PCC 6803 compared to control (1g).**

3 Objective A: Dynamics of cell under clinorotation

3.1 Dominant forces acting on a cell under clinorotation

A bacterial cell suspended inside a fluid suspension will experience various forces due to motion of fluid, gravity and due to rotation of it's frame of reference. The forces acting on the bacteria, namely, are - viscous drag, centrifugal force and gravitational force. Other forces such as Coriolis force can be ignored cause their effects are negligible compared to the forces listed here.

3.1.1 Gravitational force

Bacterial weight is a consequence of gravity. Weight of an object is the normal force that acts on it due to contact forces inside a gravitational field. Inside a clinostat a bacterial cell will fall under the influence of gravity but it will not have weight until it either (1) attains terminal velocity or (2) sediments. The bacteria will be weightless when it neither sediments nor attains terminal velocity or both.

Thus, if only gravitational force were acting on the cell, it is possible to nullify the normal weight vector by reorienting it after certain intervals over a period of time. This will prevent its sedimentation. The rotation results in the cell falling in a slightly curved path which over a complete period (of rotation) forms a circle.

But a small weight will still be registered due to the viscous force. The cell will be falling at an angle due to clinorotation, hence, the gravitational force will be,

$$\boxed{F_g = mg} \tag{7}$$

In vector notation, we can express above equation as follows,

$$\mathbf{F_g} = m\mathbf{g} = (mg\cos(wt), mg\sin(wt))$$

where, w is the angular speed.

3.1.2 Centrifugal force

A rotating frame is an accelerated or non-inertial frame of reference. This is due to the fact that even though it rotates at a constant speed, the direction of velocity vector is constantly changing too. Hence, a clinostat is also an accelerated frame of reference. This means that pseudoforce(s) will be introduced in a clinostat, namely, centrifugal

force among others.

Inside a clinostat, centrifugal force tends to push the cell away from the centre (axis of rotation). When the clinostat is rotating, the cell traverses a closed circular path. But centrifugal force tends to push the cell a little further with every rotation so that its path appears like a spiral when observed for a considerable period of time (see figure below). This result can be thought of as if the center of the cell's circular trajectory has moved away from the rotation axis (center of clinostat).

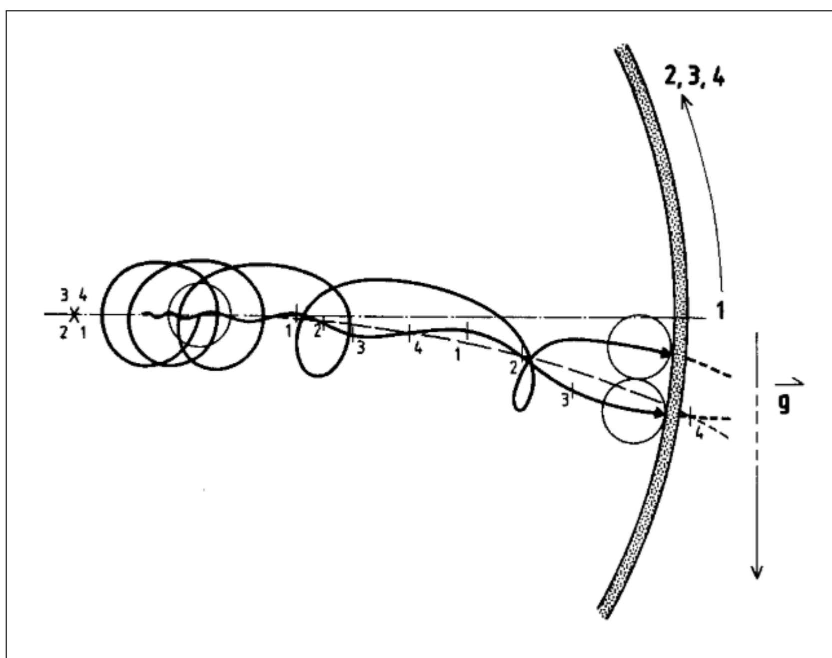

Figure 7: Trajectory of a particle in a clinostat under a dominant centrifugal force. Image Source: Briegleb (1992) [17]

The centrifugal force is dependent on the rotation (angular) speed,w, and distance from rotation axis, r. Mathematically, we express it's magnitude as follows,

$$F_c = mw^2r = \frac{mv^2}{r} \tag{8}$$

3.1.3 Viscous force

A bacterial cell inside cell suspension can be thought of as a sphere falling inside a fluid. Assuming the fluid is uniform, gravity will pull this sphere (cell) downwards while the fluid particles below it will oppose this fall. As a result, the cell will experience opposition to its motion and this opposing force will be proportional to its velocity (let's call it v_{sed}, for sedimentation velocity).

This opposing force is given by Stokes law which states that the viscous force for an object falling in a fluid is given by,

$$\boxed{F_v = 6\pi\eta R v_{sed}} \tag{9}$$

where, η is the viscosity of the fluid and R is the average radius of the bacterial cell.

3.2 Parameters describing motion of a cell during clinorotation

3.2.1 Velocity of sedimentation

The velocity at which the cell falls inside the clinostat can be obtained by equating gravitational force to the opposing viscous drag. The velocity thus obtained will be sedimentation velocity at which the cell will fall inside the clinostat when it rotates. Equating the two forces imposes a balancing condition that's satisfied when the cell has travelled enough distance in the fluid. But if the fluid in which the cell is present has viscosity greater than that of the cell, it attains this terminal velocity earlier than it would in a less viscous fluid.

$$mg = 6\pi\eta R v$$

$$\frac{4\pi R^3}{3}(\rho_c - \rho_f)g = 6\pi\eta R v$$

which simplifies to

$$\boxed{v_{sed} = \frac{2gR^2(\rho_c - \rho_f)}{9\eta} \quad \text{(in cm/s)}} \tag{10}$$

where, ρ_c and ρ_f denote densities of the cell and fluid respectively.

3.2.2 Radius of circular trajectory

Under no rotation ($w = 0$), the cell will fall down in a straight line and settle at the bottom of the vessel containing it. It will fall freely within the liquid at speed v_{sed}. Whereas under rotation ($w \neq 0$), the cell follows a circular trajectory, whose radius is given by,

$$r = \frac{v_{sed}}{w}$$

Substituting for v_{sed} in above equation yields,

$$\boxed{r = \frac{2gR^2(\rho_c - \rho_f)}{9w\eta} \quad \text{(in cm)}} \tag{11}$$

3.2.3 Centrifugal displacement

Rotation of the clinostat affects the cell's *fall* due to centrifugal force acting on the cell. This force pushes the cell further from the center of the clinostat (rotation axis). Thus there is a separation between the centers of the previous and the next circular trajectory of the cell (see figure 8).

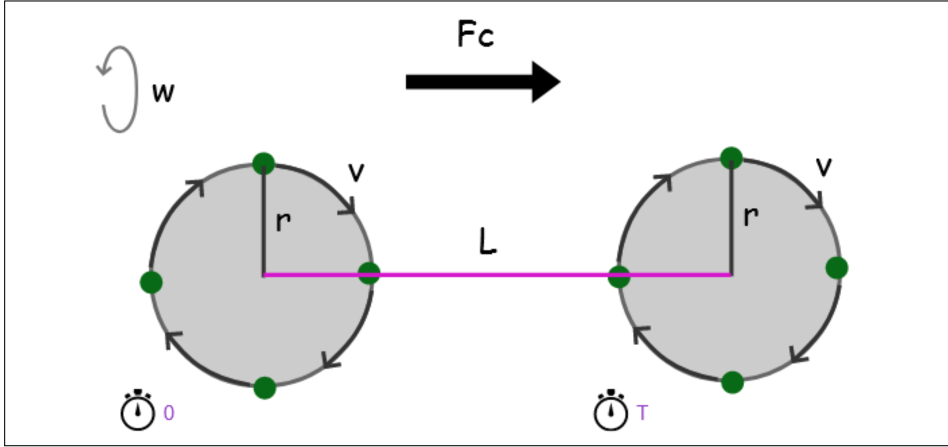

Figure 8: Centrifugal displacement. Here, the green circle denotes a cell and the numbers label position of the cell during a complete rotation of clinostat. w is rotation speed. T is labeled for required by centrifugal force to displace the cell by distance L (shown by violet line).

We can use RCF (relative centrifugal force) to find the centrifugal acceleration as follows,

$$RCF = \frac{F_{cf}}{mg} = \frac{ma}{mg} = \frac{a}{g}$$

Also, $RCF = 1.118 \times 10^{-5} w^2 L$. Therefore,

$$a = 1.118 \times 10^{-5} w^2 L g$$

The velocity (at which the distance from the center increases due to F_c) from above equation can be written as[2] ,

$$v' = 1.118 \times 10^{-5} w^2 L v_{sed}$$

Thus we can write, $v' = \frac{dL}{dt}$ to obtain a differential equation,

$$\frac{dL}{dt} = 1.118 \times 10^{-5} w^2 L v_{sed} = \frac{2.484 \times 10^{-6} w^2 L g R^2 (\rho_c - \rho_f)}{\eta}$$

With the initial condition that at $t = 0$, $L = L_o$ we can solve above differential

equation, to obtain,

$$L(t) = L_o \exp\left\{\frac{2.484 \times 10^{-6} w^2 g R^2 (\rho_c - \rho_f) t}{\eta}\right\} - L_o \quad \text{(in cm)} \tag{12}$$

Above equation tells us the us how far the cell will be, after time t, from the center (rotation axis) given that it was at L_o cm away at $t = 0$.

3.2.4 Minimizing centrifugal force

Since centrifugal force tends to displace the rotating cell. Following the approach used by Dedolph (1971) we now derive the expression for optimum angular speed for a bacterial cell. Within a time interval t, this centrifugal displacement is given by (12). This area (which is essentially two half-circles and a rectangle) can be used to obtain a minimizing condition for centrifugal displacement. The reason to obtain this condition is to choose appropriate settings to minimize the effects of centrifugal force on the cell.

$$\text{Area} = A = \frac{\pi r^2}{2} + \frac{\pi r^2}{2} + (2r)(L) = \pi r^2 + 2rL$$

Using (11) and(12), we get,

$$A = \pi(\frac{2p}{9w})^2 + 2\frac{2p}{9w}(L_o(\exp\{cw^2pt\} - 1))$$

where $p = \frac{gR^2(\rho_c - \rho_f)}{\eta}$ and $c = 2.484 \times 10^{-6}$.

To minimize above equation with respect to the angular speed we first simplify it using the approximation

$$\exp\{cw^2pt\} - 1 = 1 + cw^2pt + \frac{(cw^2pt)^2}{2} + \ldots - 1 \simeq cw^2pt$$

Then A reduces to the following equation,

$$A = \frac{4\pi p^2}{81w^2} + \frac{4p^2w^2tL_oc}{9}$$

Differentiating it w.r.t w and setting the differential to zero we get

$$\frac{8\pi p^2}{81w^3} = \frac{4p^2L_oct}{9}$$

Which finally reduces to give a condition on the angular speed, given as follows,

$$w \simeq \frac{283.567}{(L_o t)^{1/3}} \quad \text{(in rpm)} \tag{13}$$

3.3 Case study: Parameters describing the motion of cyanobacterial cell during clinorotation

So far we have described the forces acting on a cell under clinorotation and described the important parameters required to describe the motion of this cell. Now, we will evaluate these parameters for a typical cyanobacterial cell. For a *Synechocystis* PCC 6803 cell suspended inside BG-11 media, we can assume following values to calculate optimal w, and use that to calculate v_{sed}, r and L. Since the cell suspension will be under clinorotation for 5 days, we choose $t = 5$ days or 7200 mins.

We assume that the density and viscosity of BG-11 media is approximately equal to that of deionized water[20]. For convenience all quantities are expressed in C.G.S units. The results are discussed in next section.

- Density of BG-11 media: $\rho_f = 1\ g/cm^3$ (assumption)
- Average radius of *Synechocystis* PCC 6803 [18]: $R = 1.035\ \mu m$
- Density of cytoplasm [19]: $\rho_c = 2\ g/cm^3$
- Viscosity of BG-11 media [21] $\eta = 0.057$ poise
- Distance from axis of rotation: $L_o = 1.5\ cm$

Substituting value of L_o and $t = 5$ days $= 7200$ mins in (13), we get the optimal rotation speed at which centrifugal effects will be minimal,

$$\boxed{\text{Rotation speed}: \quad w \simeq 13\ rpm} \tag{14}$$

From equations (10), (11) and (12), we get,

$$\begin{array}{rll} \text{Sedimentation velocity}: & v_{sed} & = 4.1 \times 10^{-5}\ cm/s \\ \text{Radius of trejectory}: & r & = 3.59 \times 10^{-6}\ cm \\ \text{Centrifugal displacement}: & L(5\text{ days}) & = 8.35 \times 10^{-4}\ cm \end{array} \tag{15}$$

Inside a clinostat, three forces are dominant. We saw an overview of each of these in the previous subsections and derived some important theoretical results in the subsection following that. We obtained a rotation speed of 13 rpm which, more or less, agrees with the value used in most microgravity experiments that use clinostats. For instance, Xiao et. al (2010) used a rotation speed of 10 rpm. The cells under study belonged to *Microcystis aeruginosa*, another Cyanobacterial species.

Fixing the value of rotation speed as $w = 13$ rpm, we obtained other quantities. Namely, sedimentation velocity, radius of trajectory and centrifugal displacement. The sedimentation velocity turned out to be 0.41 microns per second. This means that in one second the cell traverse a distance which is roughly half of its diameter. This makes sense if we look at the radius of this circular trajectory which is approximately 0.03 microns. This value is around 3% of the cell's radius.

The centrifugal displacement, on the other hand, is considerably large compared to the cell size. This value is about four times the cell size. This, too, makes sense considering the period of exposure which was 5 days. This problem has been briefly discussed in [2] which proposes that for long time exposure, a RPM should be employed.

3.4 Inference obtained from above discussion

When the clinostat is at rest i.e. when $w = 0$, any cell inside it will fall under the effect of gravity. This is similar to a sphere falling inside a viscous medium (see figure below). This kind of motion is very similar to free-fall except at some point, the cell will attain a constant speed and it will experience weight. It should be noted that terminal velocity is always greater than or equal to sedimentation velocity.

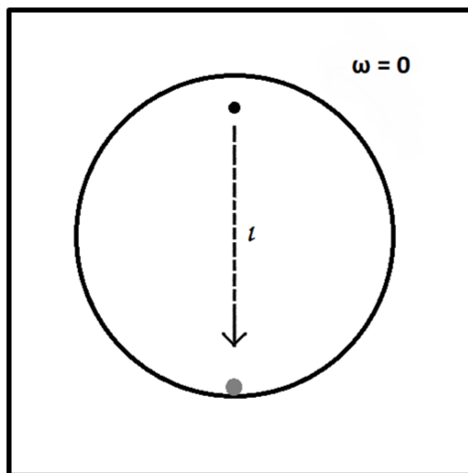

Figure 9: Sedimentation of cell under 1g.

When the cell reaches the periphery of the clinostat container, it will experience contact forces like friction that will act as a normal vector and thus, the cell will experience weight. Hence, during it's fall it experiences weight at two points - one when it attains terminal velocity and another when it sediments.

What a clinostat succeeds in doing is prevent the cell from experiencing weight at both these points by avoiding them. And does this by rotating the cell's frame of refer-

ence. When the clinostat is rotating at a constant angular speed $w \neq 0$, the fall of this cell *curves*. This traps the cell in a circular loop that prevents its sedimentation (see figure below). Equations (10) and (11) describes velocity of sedimentation and radius of trajectory for a cell respectively. These loops lead to an interesting conclusion that if the radius of these loops is somehow reduced to zero, the cell will be floating in the liquid without ever attaining terminal velocity. It will be practically weightless.

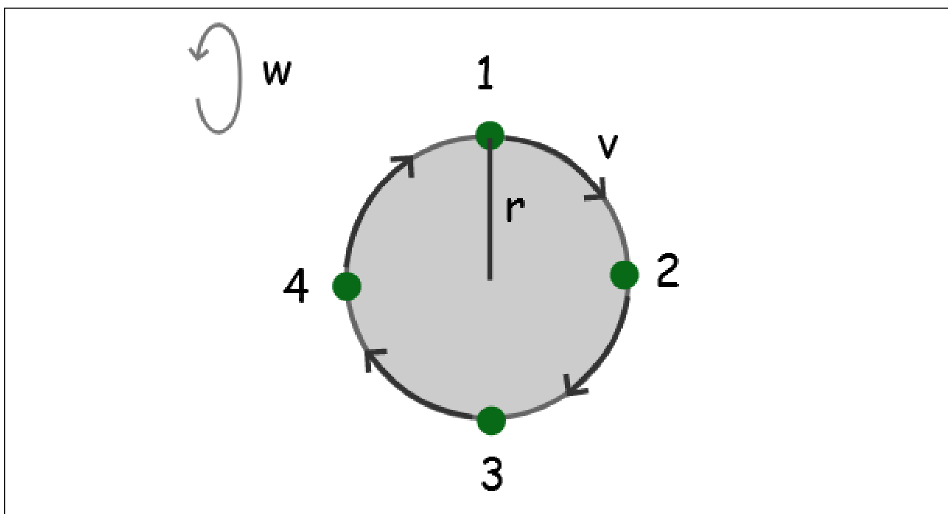

Figure 10: Motion of cell in a clinostat. Here, the green circle denotes a cell and the numbers label position of the cell during a complete rotation of clinostat. w is rotation speed.

Over a complete rotation, the cell will remain in this loop, without further falling or sedimentation. Thus, a clinostat is able to achieve weightlessness with the interplay of gravitational and viscous forces.

But there's one more force, which unlike these two forces, has a negative impact on the overall experiment. That force is centrifugal force. This force is a simple consequence of the fact that the frame of reference under consideration is an accelerating frame. Centrifugal force is a pseudoforce among other forces such as Coriolis force [17].

Centrifugal force in this case tends to push the cell towards the periphery of the container (see figure 7). An addition of a third force means that there will be unbalanced reactionary forces acting on the cell. These will be sensed as weight by the cell. Hence, minimization of centrifugal force is important. This can be done by selecting an optimum rotation speed. Rotation speeds higher than an optimum rotation speed will tend to increase centrifugal forces inside the clinostat.

Equation (12) shows that the centrifugal displacement depends on the initial dis-

placement, rotation speed and exposure time. Thus, it is important to choose these three quantities wisely to keep the impact of centrifugal force to a minimum.

3.5 Experiment: Calculating sedimentation velocity

3.5.1 Aim

To determine the sedimentation velocity of a cell under media of different viscosities

3.5.2 Purpose of experiment

A clinostat simulates microgravity by preventing sedimentation of a cell. Sedimentation of a cell depends on the viscosity and density of the medium in which it is suspended. By studying the relation between how fast cell sediments and the viscosity of the medium, we can make better choices of cultural media in which these cells are suspended. This is important because important quantities like radius of trajectory for a cell depend upon this velocity.

3.5.3 Objectives

1. To prepare salt solutions of various concentrations - 0M, 2.59M and 3.77M - and determine corresponding viscosities and densities.

2. To verify the validity of the sedimentation velocity relation for a sphere dropped inside a viscous fluid.

3.5.4 Apparatus

A transparent cylindrical vessel (diameter = 10 cm, height = 20 cm), a spherical bead (diameter = 1 cm), a mechanical balance or a weighing machine, stopwatch, water and table salt.

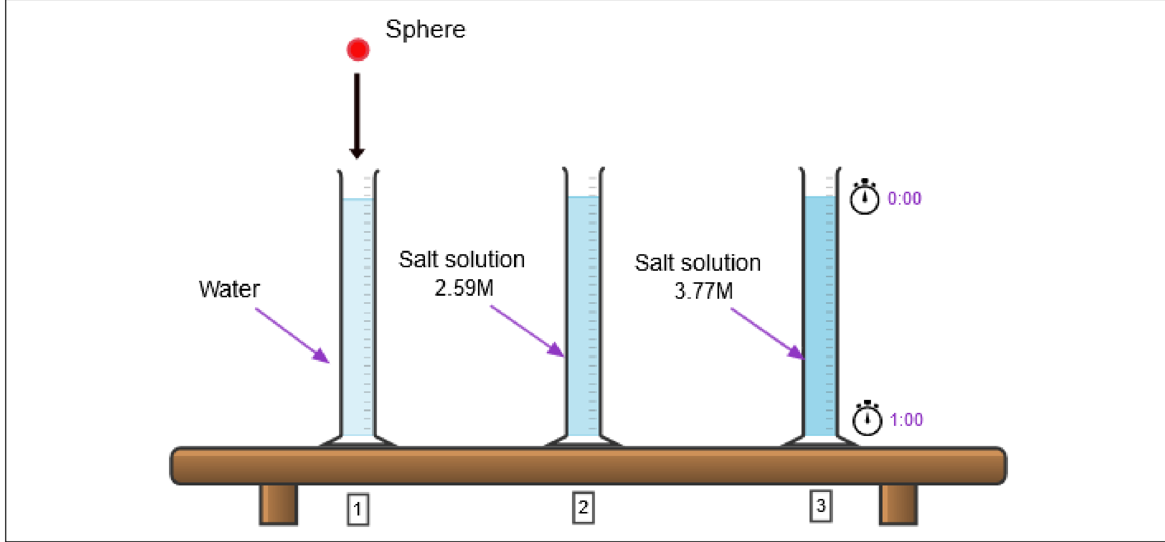

Figure 11: Experimental setup and pictorial summary of the experiment

3.5.5 Mathematical relations

1. Falkenhagen's relation [22] to determine viscosity of a solution:

$$\boxed{\eta_{sol} = (1 + 0.0062\sqrt{c} + 0.0793c + 0.008c^2)\eta_{water}}$$

   where $\eta_{water} = 0.0089$ poise (at 25°C) and c is concentration of salt.

2. Sedimentation velocity (theoretical):

$$\boxed{v_{sed} = \frac{2gR^2(\rho_s - \rho_f)}{9\eta_{sol}}}$$

   where ρ_s is density of sphere (1.1 g/cc) and ρ_f is density of solution.

3.5.6 Method or procedure

1. Determine the volume of cylindrical vessel. Fill the vessel with water till the marked position. Weigh the vessel and determine the mass of water contained in it. Drop the sphere and measure the time required for it to travel 15 cm. Repeat this few times and calculate average time. Calculate density of this water contained in the vessel.

2. Add salt 250g salt to one litre water to prepare a solution of salt concentration 3.77M [Solution 2]. Repeat the procedure done above for length 11 cm. Calculate mass, volume and density.

3. Add 520 g water to above solution. This will reduce the concentration to 2.59M [Solution 1]. Repeat the velocity measurement for length 15 cm. Calculate mass,

volume and density.

4. Calculate viscosities for each of the two solutions and water using Falkenhagen's relation. Using the formula, calculate sedimentation velocity and compare with the observed value for velocity.

3.5.7 Observations

Quantity	Water	2.59M salt solution	3.77M salt solution
Length travelled (cm)	15	15	11
Average time (s)	0.97	1.88	2.53
Calculated velocity (cm/s)	15.46	7.98	4.35
Mass of solution (g)	1600	1760	1240
Volume of solution (cc)	1570	1646	1135
Density of solution (g/cc)	1.02	1.06	1.09
Viscosity (poise)	0.0089	0.013	0.011

3.5.8 Results

Quantity	Water	2.59M salt solution	3.77M salt solution
Density of solution (g/cc)	1.02	1.06	1.09
Viscosity (poise)	0.0089	0.013	0.011
Observed velocity (cm/s)	15.46	7.98	4.35
Theoretical velocity (cm/s)	489.3	197.9	41.3

4 Objective B: Growth characteristics of *Synechocystis* under altered gravity

4.1 Growth characteristics

Materials:
BG-11 media is used as a growth media for *Synechocystis* PCC 6803. Then environment will be controlled with constant illumination of 1200 lx, and temperature 25^oC.

Treatment:
SMG - Duration: 5 days, g-value: 10^{-3}g
Hypergravity - Duration: 10 min, g-value: 500g-5000g

4.1.1 Growth curve

Growth curve (see figure 12) is basically a plot of optical density (OD 750) versus time (in days). This curve is characterized by various phases which indicates different growth phases of a bacteria. These phases are:

- Lag phase where cells are adjusting to their environment, without dividing.
- Exponential phase where cells divide exponentially.
- Stationary phase where the cells stop dividing; number of dividing cells is equal to number of dying cells.
- Death phase where cells exhaust their nutrients and start dying.

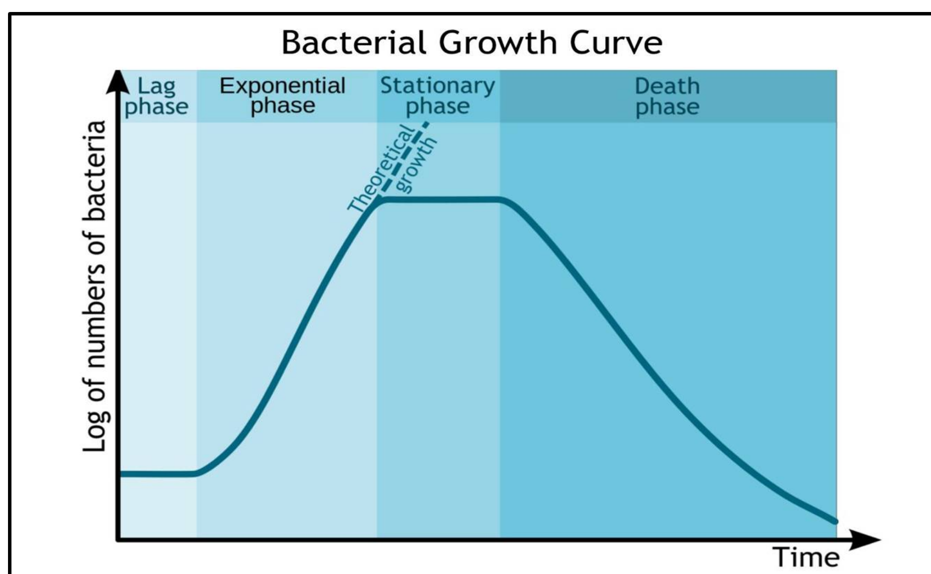

Figure 12: Bacterial growth curve. Image source: Bailey (2021) ThoughtCo

Method: Two capillary tubes containing the cell suspension, one control and another test, are prepared and initial optical density (at 750nm) of each is recorded using a UV-Vis spectrometer (latter measurements are also done using the same equipment). Control group is kept in normal gravity (1g). Test groups are kept under SMG and hypergravity conditions and after each day 1 ml of sample is taken to measure the optical density at 750 nm. And OD for this sample is measured and noted. Obtained OD is plotted against time to get a growth curve.

4.1.2 Cell viability

Cell viability is a qualitative measure which indicates the number of cells that are metabolically active. It is a measure that helps us determine whether the environment is favourable to the cell growth or not. In short, viability is a measure of cells' overall health.

Method: Control group is kept in normal gravity (1g). Test groups and kept under SMG and hypergravity conditions and 1 ml of sample is taken at the end of exposure period. Liquid dye assay (biochemical assay) is used to determine cell viability. Dye assay stain cells that are metabolically active by a certain colour (e.g. MTT dye assay stains healthy cells purple whereas dead cells don't get any stain).

4.1.3 Cell morphology

Cell morphology essentially means shape, size and appearance of a cell. Morphology of a cell can determine how cells interact with their surroundings and these interaction, in turn, determine their function. For instance, reduction in cell size will reduce surface area of cells which might affect drug interaction and how transport across membrane occurs.

Method: Control group is kept in normal gravity (1g). Test groups are kept under SMG and hypergravity conditions and 1 ml of sample is taken at the end of exposure period. Cells were immediately fixed with 5% glutarldehyde. SEM (scanning electron microscope) is used to obtain images of cells to observe the changes in control and test groups.

4.2 Studies under simulated microgravity and hypergravity

Condition	Organism	Simulator used	Environmental conditions	Parameters studied	Results	References
Simulated microgravity (10^{-3}g)	*Microcystis aeruginosa*	Rotary cell culture system (RCCS)	20°C with 12:12 hour light/dark illumination; BG-11 media	• Cell number • Chlorophyll fluorescence • Pigment concentration • Nutrient uptake • Microcystin	• Decreased • Decreased • Increased • Increased • Increased	Xiao et al. (2010) [13]
Space flight(10^{-4}g-10^{-5}g)	*Anabaena siamensis*	Miniaturized bio-reactor (200 ml) aboard a retrievable satellite	20°C with 12:12 hour light/dark illumination; BG-11 media	• Growth rate • Chlorophyll fluorescence • Chlorophyll content • Oxygen production rate • Nitrogenase activity	• Increased • No change • Decreased • Increased • Increased	Wang et. al (2006) [14]
Hypergravity (90g, 180g, 40000g)	*Synechocystis* PCC 6803	Centrifuge	25°C with continuous illumination and aeration; a modified Allen and Arnon media	Dehydrogenases activity	Slight reduction (90g & 180g); Decreased (40000g)	Erdmann et al. (1997) [15]
Simulated microgravity (10^{-2}g)	*Synechocystis* PCC 6803	2D clinostat	25°C with continuous illumination; grown on solid agar	• Photochemical quantum yield • Chlorophyll content • Total protein content • MDA content • Catalase Activity	• Decreased • Increased • Increased • Increased • Increased	Zhang et. al (2012) [16]

5 Discussion and conclusion

A. Dynamics of cell under clinorotation

In section 3, we saw how the three dominant forces - gravitational, viscous and centrifugal - together give rise to the circular motion of a cell under clinorotation. This motion is mainly characterized by four parameters - sedimentation velocity, radius of trajectory, centrifugal displacement and angular frequency (rotation speed).

Given rotation speed and sedimentation velocity we can determine other two parameters and thus are in a position to describe the motion of a cell under clinorotation. A cell can be thought of as a sphere suspended inside a fluid. This cell will fall under the gravitational force at a speed given by sedimentation velocity relation. If the suspension fluid (culture media) has high viscosity, the cell will fall slowly compared to falling in a less viscous fluid. This velocity, along with rotation speed, also determines the radius of trajectory (r) for the cell inside a clinostat ($v = rw$, where w: rotation speed). A smaller radius will mean that the cell would be practically floating and in a way, weightless.

Viscosity of media not only determines sedimentation velocity, it also indirectly influences the radius of trajectory. Thus, with the knowledge of viscosity of media (and hence, sedimentation velocity), we can better predict the motion of cells inside a clinostat. And to study how sedimentation velocity varies with viscosity of the media, we performed an experiment (see 3.5). From that experiment we arrive at the following conclusions:

1. We observe a decreasing trend in sedimentation velocity (for both observed and theoretical velocity) with increase in viscosity of solution which verifies the inverse dependence of sedimentation velocity on viscosity.

2. The differences between the observed and theoretical values of sedimentation velocity can be possibly explained by the observed density of water. Since the density of water used (regular or non-distilled water) is not 1 g/cc, the viscosity of water that has been assumed (0.0089 poise) is actually greater than that of pure water and hence, the theoretical velocity should be lesser than the values we obtained. Thus, assumed value for viscosity of water is one possible source of error.

B. Growth characteristics of cyanobacteria under SMG and hypergravity

We reviewed four papers ([13], [14], [15] and [16]) which studied various growth characteristics and related parameters of different cyanobacterial species under simulated microgravity and hypergravity (see subsection 4.2). Growth rate, chlorophyll fluorescence, photosynthetic pigment concentration, activity of dehydrogenases, nitrogenase, catalase etc were a few of the parameters that were studied.

Growth rate was observed to have been reduced under SMG, whereas in a space flight increased growth was observed compared to control. These differences in results can be attributed to the kind of devices used since the device which simulated microgravity showed a decreasing growth rate and the bio-reactor aboard a satellite showed an increase in cyanobacterial growth.

Variation was observed in chlorophyll fluorescence which denotes photosynthetic efficiency. But no substantial claim can be made regarding the trend of chlorophyll fluorescence and hence, it remains inconclusive whether SMG had any effect on the photosynthetic efficiency. There was variation in photosynthetic pigment concentration but no linear change was observed. Increase in production of oxygen was observed in one study, however, further studies are needed to derive any conclusion in this regard.

Under hypergravity, insignificant reduction was observed in dehydrogenase activity. But at much higher g-values (greater than 40000g) significant decrease in dehydrogenase activity was observed. And since, dehydrogenase is related to the cell viability, hypergravity might negatively affect cell viability.

Finally, activities of various enzymes (such as catalase, MDA, nitrogenase etc), protein content and cyanotoxins showed a definite increase under simulated microgravity. Increase in enzymatic activity was observed even under different devices and slightly different environmental conditions. Thus, we can infer that simulated microgravity increases enzyme activity in cyanobacteria.

In conclusion, we can say that altered gravity (simulated microgravity or hypergravity) does affect growth of cyanobacteria. Though more studies are required to establish the growth trend, with certainty, for various characteristics (such as enzymatic activity, pigment concentration, etc) of cyanobacteria under SMG or hypergravity.

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