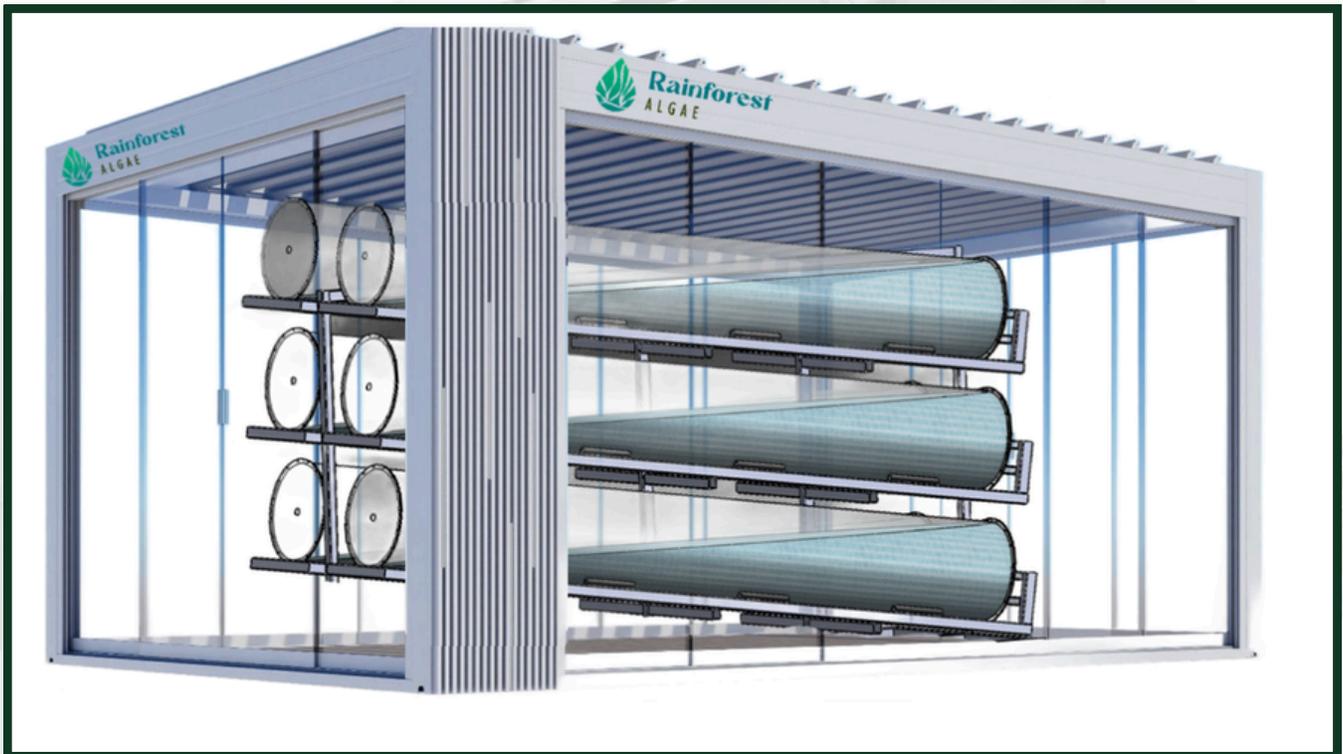




Rainforest
ALGAE

PRODUCT TECHNICAL SPECIFICATIONS





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Rainforest Algae Corp. has been working to develop the most advanced bioreactor system available, taking advantage of the extraordinary advancements in culture mixing and mass transfer of gases between the liquid and gas phases that are essential to optimizing reaction yields whether these involve chemical, photosynthetic or fermentation reaction kinetics. To reduce algae cultivation operating costs there it is possible to integrate renewable energy sources. The sun offers us free solar energy to promote algae growth. It is important that where possible, **photobioreactors (PBRs)** should utilize this energy when it is available. This is why Rainforest promotes the use of an enclosure system for smaller commercial systems and the use of greenhouse enclosures for larger systems.

Whether dealing with cellular agriculture, algae, fermentation cultures, process, aquaculture or wastewater treatment applications, the Rainforest reactor systems are rapidly scalable from 2L through to 100 m³ and potentially to 1,000 m³ culture volumes. Reaction schedules can be set for batch, fed-batch (semi-batch), or continuous operations.

REACTOR VESSELS

All Rainforest Algae reactor vessels have cylindrical bodies, offering a high surface-area-to-volume ratio and facilitating uniform light penetration through the culture. The reactor vessel rotation speed is controlled by the CMS (described below) and provides smooth, laminar or gentle turbulent flow which reduces shear stress, preventing damage to shear-sensitive algae or cyanobacteria while provided enhanced mass gas transfer and medium mixing. Furthermore, all reactor vessels are sealable to ensure aseptic reproducible culture growth with option for eight (8) or more ports for mounting various components (e.g. probes, tubes, etc).

The vessels may all be **Cleaned In Place (CIP)** or in the case of both the Minnow and Chinook PBRs, the associated vessels are of a size that allows them to be autoclaved for sterilization. In larger reactor vessels CIP would involve protocols to guarantee asepsis through washing / rinsing / disinfection cycles controlled by the software Control and Monitoring System (CMS) described below. Peristaltic pumps are used to deliver aseptic cleaning agents through tubing and the reaction vessel for cleaning.

Recommended Aseptic Cleaning Agents (Safe)

- Isopropyl Alcohol (IPA): Maximum 30% concentration for standard acrylic.
- Hydrogen Peroxide: 3% solution is generally safe (e.g. Lysol brand).
- Diluted Bleach (Sodium Hypochlorite): A mixture of 1/3rd cup of bleach per gallon of water (approx. 1:50 dilution, or 0.5%–1% NaOCl) is generally safe for disinfection.
- Mild Soap & Water: The safest method for daily cleaning.
- Acids: Diluted (5%–10%) acetic acid (vinegar) is generally safe for removing water spots

Chemicals to Avoid (Acrylic Damage Risk)

- ACRYLIC should NEVER be steam sterilized (autoclaved) as it degrades at temperatures above 50°C - 80°C.
- High-Concentration Alcohols: >30% IPA for uncoated acrylic.
- Ammonia-based cleaners: (e.g., standard Windex).
- Strong Solvents: Acetone, benzene, gasoline, lacquer thinners, carbon tetrachloride.
- High-Alkaline Cleaners: Strong degreasers (pH 13-14), concentrated sodium hydroxide
- Strong acids: fuming nitric acid, strong hydrochloric acid.
- Abrasives: Scouring pads or gritty cleaners

Key Considerations for Aseptic Procedures

- Contact Time: Limit contact time to minimize degradation. Rinse with water immediately after disinfection.
- Environmental Stress Cracking: Acrylic is susceptible to cracking when exposed to cleaners while under stress (e.g., tight screws, bent sheets).
- Hard-Coated Acrylic: If using 70% alcohol or harsh disinfectants, verify if the material has a specialized "hard coat" designed for chemical resistance.
- Application: Always test cleaners on an inconspicuous area first.

Cleaning Procedure

- Clean: Remove organic matter with mild soap and water.
- Disinfect: Apply approved agents (e.g., 3%)
- Rinse: Thoroughly rinse with RO water to prevent residue buildup.

For the Rainforest Algae **Minnow** and **Chinook** reactor vessels, there is an option to choose between a **food grade PET plastic construction and borosilicate glass**. Borosilicate glass offers higher light transmittance, chemical inertness, corrosion resistance and thermal shock resistance that withstands autoclaving for sterilization and maintaining aseptic conditions. Conventional round bottom vessels aid in effective mixing, reduce shear stress and improve gas transfer.

Larger and scalable versions of the Rainforest Algae reactors all rely on acrylic cylindrical tubes that are resistant to chemicals and detergents with smooth surfaces to minimize fouling / accumulation of biofilm and facilitate cleaning. Although the Rainforest Technologies Product Brochure provides culture capacity of medium volume (**420L, 1250L** and beyond), clients can actually order the configurations best suited to their own operations. Although there are applicable dimensional constraints, clients may choose to achieve a certain volume / medium capacity that is of interest to them and then order their reactor vessels at a length, diameter and number of reactor tubes that will work best for them. This can be readily calculated by determining the volume of a given cylindrical reactor vessel and dividing by two. The reactor's design relies on only one half of the volume being the medium and that the vessel is inclined to promote efficient three-dimensional mixing and optimizing mass gas transfer. As you scale, having multiple reactor vessels helps to mitigate the potential of costly culture upsets and the opportunity to re-bounce back to full production quickly should you lose the culture in a single vessel. Additionally you have the option to run more than one culture at a time. This arrangement also facilitates vertical stacking.

RAINFOREST CONTROL MODULE SYSTEM (RF-CMS)

The Control Module System (CMS) is a powerful software platform and an integral component of every Rainforest reactor system. The system can monitor multiple parameters (e.g. pH, pressure, dissolved oxygen), process, graph and log data for export and automatically control multiple components based on operator and sensory input data. The user interface (UI) for the CMS may be found on touchscreens associated with the various reactor systems. The Minnow is the only reactor system that does not have its own touch screen. This must be ordered separately and is designed to control up to 6 Minnow reactors simultaneously. Given the WiFi capability of the CMS, this system has been designed to also work on desktop/laptop computers and devices like smart phones, tablets, or iPads.

All sensors integrated into the CMS are able to be monitored with data being logged in real-time and may be used to control specific system parameters within defined ranges or schedules. The CMS continues to be advanced to not only optimize yields but move the technology toward a fully automated platform. Reactor vessels are able to be sealed and can be cleaned in place (CIP) to be able to grow contamination free cultures. Depending on reaction requirements, the system can be configured with controllable lighting systems, temperature, gas exchange, nutrient additions, harvesting pumps, parameter sensors, etc. The CMS may be configured to respond to reaction kinetics and / or AI / ML algorithms.

Light intensity control. Light intensity may be adjusted through the CMS. **Photosynthetic Photon Flux Density (PPFD) measurements ($\text{mol}/\text{m}^2/\text{s}$)** are provided at the photobioreactor's inner surface. Likewise, automated daytime light-dark cycles may be set through within the CMS.

pH Control. All Rainforest Algae reactor system include pH probes. pH may be monitored and controlled through the CMS. Once a pH set point and range has been entered, the system will automatically adjust pulsing duration and frequency to control the CO_2 pulsing to hold the pH set point that has been established. Alternatively pH control may be provided through injection of solutions delivered by way of peristaltic pumps.

Temperature control. Temperature may be controlled through the CMS is impacted primarily by light intensity and ambient temperatures. A thermocouple is placed into the culture medium and connected to the CMS. Operating temperature set point and temperature range can be set between 5°C to 40°C . Circulation of heated / cooled liquid flows through a **PBR "jacket"** is automatically controlled by the CMS to be able to hold the temperature of the culture medium at the SET POINT established by the operator.

CULTURE MIXING

The culture medium is effectively mixed by the rotating PBR tube. Rotation speed of the PBR tube may be set and changed on the CMS to increase / decrease agitation / stirring.

AERATION

In the Rainforest bioreactors, although it is possible to arrange for **gas diffusion / dispersion** using bubbles, this is generally not required. Gases are injected into the head space of the tubular reactor and it is the **effective mixing of the media and rapidly renewing wetted surface area of the rotating reactor tube that enables for extraordinary surface area for the efficient mass gas transfer to take place.**

The rate of gas exchange has to do with temperatures, reactor pressures, partial pressures of individual gases in both the bubbles and the medium, mixing of the media and also surface area of the bubbles. Each of these parameters may be controlled through the CMS.

This spinning reactor tube with the effective mixing of the medium also reduces the potential of fouling within the reactor vessel and this can also significantly reduce foaming challenges.

In conventional reactor systems, aeration, the mechanism whereby gas transfer from a gas phase into a liquid medium is achieved by the dispersion and release of gas fine bubbles from the bottom of a reactor vessel which rise through the medium while exchanging gases between the dissolved gases in the liquid medium and the gases in the bubbles. As the bubbles rise they also assist in mixing of the medium. A major cause of foaming in cultures is related to / caused by the extensive dispersion of the bubbles throughout the column of the reactors. Foaming is significantly reduced compared to other conventional reactor systems in the Rainforest Algae reactor vessels.

FOAM CONTROL

The Rainforest Algae reactor vessel design reduces the need for foam control. Operational issues linked to foam formation including clogged filters, contamination, reduced working volume, and decreased gas transfer rates is significantly reduced in the Rainforest Algae reactor vessels. This is due to the fact that diffusion aeration (bubbling the gas into the medium) and mechanical stirring mechanisms are not present and the rotating reactor system design minimizes shear forces that can lead to foaming. However, in the event of foam formation often associated with surfactants and proteins released by organisms, the addition of standard antifoaming agents may be introduced via peristaltic pumps.

NANOBUBBLES (NB)

The addition of **Nanobubbles (NB)** offers a further step that can be taken to ensure an even faster gas exchange to take place. Mass gas transfer in many reactions remains a key limitation in reaction kinetics and the ability to achieve higher yields of desired products.

Nanobubbles are bubbles that have a diameter of 10^{-9} m. These are bubbles that are not able to be seen in the visible spectrum because their diameters are shorter than a wavelength of light. So, you will not find them even with a light microscope. What is unique and important about them is that they are "meta-stable". They tend not to coalesce or join other bubbles and therefore tend to be neutrally buoyant and do not "pop" to the surface of the medium.

That means that they can become the “sponge” to transfer and exchange gases. It is possible to have 10^6 through 10^8 and potentially 10^9 NB/ml in solution and are possible to be created with nanobubble generators. If you add up the surface area of the millions of NB, it is possible to have a much higher total surface area for the mass gas transfer to take place while also super saturating the medium with the gas.

Rainforest Algae has developed NB generators that achieve extremely high NB concentrations in support of higher reaction rates and more efficient exchange of gases within the media. Contact us to inquire about stationary or flowthrough NB generators.

CULTURE OPERATION (BATCH, FED-BATCH, CONTINUOUS)

The Rainforest Algae reactor system can be set up by the PBR operator to manage the culture as a “Batch” culture, having pre-mixed the growth medium with all of the nutrients to sustain the growth of the culture for the duration of the culture growth cycle.

The reactor can also be set to provide a “Fed-Batch” culture, a semi-open bioreactor strategy where nutrients are fed continuously or intermittently to a batch, preventing nutrient depletion and extending the exponential growth phase. This technique maximizes cell density and product yield (e.g., proteins, antibiotics) while controlling growth rates and minimizing toxic metabolite accumulation. This is accomplished through the CMS to control peristaltic pumps delivering nutrients to the culture.

GAS DELIVERY FOR REACTOR VESSELS

Each reactor system is configured with an **air pump system** drawing air through a $0.2\mu\text{m}$ filter system. The air pump system will be configured for the size and desired gas flow rate and / or gas pressure requirement for each vessel size.

For each reactor vessel size a manual air / CO_2 flowmeter (rotameter) may be set for ranges from 0.1 LPM to 30 LPM or even greater depending on the culture volume found in the reactor vessel. For delivery of gas from pressurized tanks, primary gas would be regulated at the tank to desired line pressure (to be provided by others). A gas flowmeter controls gas flow into the reactor vessel.

To automate control of a pressurized CO_2 air stream, a gas valve solenoid opens and closes based on the reactor system operator’s instructions in the CMS to pulse the CO_2 into the reactor vessel in a manner to control pH levels. An adjustable gas flow rate solenoid controlled by the CMS is also available for specialized operations.

Air Pressure Control System (APCS): Air pumps (RF-AP) working in conjunction with flow meter settings and an analog pressure gauge (RF-APG) connected to CMS enable automated control of the adjustable gas valve (RF-GVS-Adj) to maintain a constant pressure within the reactor vessel.

*** CAUTION: The reactor vessel pressure under the Rainforest Algae standard assembly configuration should NOT EXCEED 0.1 MPa (14 psi, 1 bar).**

LIQUID DELIVERY / TRANSFER FOR REACTOR VESSELS

Peristaltic pumps and control valves may be controlled by the CMS to deliver adjustable flow rates and volumes of liquids at prescribed times / intervals and with capability to both deliver and remove liquids (supply and recover) simultaneously. Upon request, flowmeters could be integrated to provide greater precision of transferred fluids.

INSTALLATION TOOL KIT

Hand tools for installation and day-to-day operations of the PBR system will be supplied along with a complement of spare parts for key operating components.

POWER REQUIREMENT: **IMPORTANT: The Beluga 1250L PBRs require a 240V / 50A connection or as many as 8 each 110-120V / 15A circuits.

RAINFOREST CONTROLS: EXAMPLES

- Schedule daytime light/dark cycles
- Control lights
 - Intensity
 - Pulsing (on/off scheduling)
 - Intensity as culture densities change (algae)
- Control culture temperature
 - Fans
 - Cooling system
 - Lights & lighting intensity / pulsing light.
- Control mixing - change the speed of the rotating reactor
- Control foaming - change speed of the rotating reactor / pulse in anti-foaming agent
- Control reactor vessel pressure