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A re-usable wave bioreactor for protein production in insect cells

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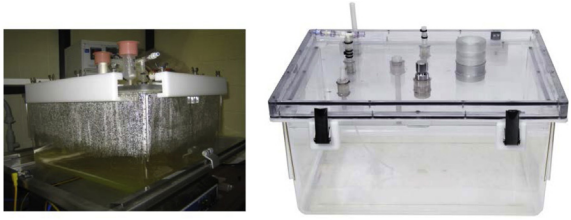
GRAPHICAL ABSTRACT



Polycarbonate bioreactor for insect cell cultivation on shaking platforms

ABSTRACT

Wave-mixed bioreactors have increasingly replaced stainless steel stirred tank reactors in seed inoculum productions and mammalian cell-based process developments. Pre-sterilized, single-use plastic bags are used for cultivation, eliminating the risk of cross-contamination and cleaning procedures. However, these advantages come with high consumable costs which is the main barrier to more uptakes of the technology by academic institutions. As an academic Core Facility that faces high demand in protein production from insect cells, we have therefore developed a cost-effective alternative to disposable wave bags. In our study we identified:

- A re-usable wave shaken polycarbonate bioreactor for protein production in insect cells achieves protein yields comparable to disposable bags.
- The advantages of this re-usable bioreactor are low costs, long life cycle, flexible configuration of accessories and convenient handling due to its rigid shape.

ARTICLE INFO

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Method details

Preparation of bioreactor

1. As a cultivation container, a polycarbonate box 500 U Eurostandard Typ IV S, TECNIPLAST, Italy, Size 480 × 375 × 210 mm, Volume 38 L, originally made for animal housing was used. The material is transparent and autoclavable repeatedly at 121 °C. The 1st Cooper model was autoclaved and used 35 times without loss of performance.
2. The lid was constructed in-house. It consists of polycarbonate at corresponding size and is sealed with a layer of rubber foam. Six metal clamps on four sides guarantee tight attachment to the base container.
3. Within the lid, seven ports with connectors to both inner and outer sides are inserted. These insertions are screw threads manufactured in-house.
   a. Two openings are standard 13.5 Pg ports for optional pH or pO_2 sensors (not used in our settings).
   b. One single wide-mouth opening of 5.4 cm inner diameter serves as air outlet, covered with C55 Silicon Cap (Hirschmann, VWR, Cat No 8905755) and protected by aluminium foil during autoclaving.
   Another four ports (one wildcard) at inner diameter of 0.5 cm have been introduced for
   c. Air inlet, sterile filtered through an Acro 50, 0.2 μm PTFE filter, (Pall, P/N 4151), also covered by aluminium foil for autoclaving.
   d. Aseptic sampling with Super Safe Sampler (Infors, Cat No 65373) and thin inside tubing (ID 2 mm, OD 4 mm), that allows to take smallest sample volumes without any risk of introducing contaminations.
   e. Medium fill via a long (outside 2.30 m, inside 24 cm) tubing, connected via stainless steel quick connectors (Stäubli, Cat No RBE06.1806/IC/OD/JE, RBE06.7806/IC/JE) to a 1 L Schott bottle, pressure balanced by an Acrodisc CR 0,45 μm PTFE sterile filter (Pall) for autoclaving.
4. Pharma-compliant platinum cured silicone tubings (Applied Critical Fluids, Cat No I-SA P60 40-24-88, ID 4.0, OD 8.8; Wall 2.4 mm) were used. The tubings are suitable for peristaltic pumps and autoclavable.
5. Bioreactor and connected Schott Bottle are sterilized by autoclaving (121 °C, 15 Ψ, 20 min). After sterilization, the Schott bottle can be replaced by the medium reservoir or starting culture eg. in a 2 L bottle to be pumped into the reactor. This port can also be used for fed-batch during cultivation.

Small and large scale insect cell culture protocols

1. Sf9 (Life Technologies) or Hi5 cells (provided by Gene Center, LMU Munich) were grown in suspension in Ex-Cell 420 (Sigma, Cat No 24420C). Cell count, viability and cell diameter were analyzed using the Vi-cell XR cell counter (Beckman Coulter). Cells were maintained at a density 1–5 × 10^6 cells/mL and 0.2–5 × 10^6 cells/mL for Sf9 and Hi5 cells respectively. Cell diameter is about 19 μm, and viability should be higher than 95%. It is important to ensure that cells are approximately those values in order to maintain high quality and reproducibility of experiments.
2. Cells were grown in shaker incubators (Infors, 50 mm rotating diameter) at 27 °C in Erlenmeyer EM or Fernbach FB glass flasks covered with Silicon Caps (Hirschmann, VWR) at the following combinations of culture volume – flask volume – shaking speed: 30 mL – 250 mL EM – 120 rpm; 150 mL – 1 L EM – 120 rpm; 200 to 400 mL – 1,8L FB – 90 rpm; 400 mL to 1 L – 5 L EM – 90 rpm
3. Baculovirus expression was performed according to the Bac-to-Bac® protocol (Life Technologies). Bacmid transfections in Sf9 cells were harvested after 3–5 days. Virus titer was determined using the SF9-ET easy titer cell line [1]. Virus was either amplified in two subsequent steps to generate Passage 1 and Passage 2 virus stocks or used to generate Baculo Infected Insect Cells (BIIC) as described previously [2]. BIICs frozen at −80 °C served both for protein expression and as virus storage. For protein expression, Hi5 and SF9 cells were adjusted to 1 × 10^6 cells/mL and infected with virus stock or BIICs at different dilutions, typically in the range 1:1000–1:10,000.