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Extended Abstract

Bioreactor-Scale Production of Infectious Laryngotracheitis Virus-Glycoprotein G using a Baculovirus/Insect Cells Culture System

Quispe Juana
Universidad Nacional Mayor de San Marcos, Peru

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Abstract:

Infectious Laryngotracheitis virus (ILTV) causes an important respiratory disease in poultry. Glycoprotein G (gpG) is a virulence factor in ILTV that was identified as a viral chemokine binding protein (vCKBP) with the ability the bind chemokine's from chickens and other species and modulate their activity. To further study the functional and structure characteristics of gpG are important to have enough quantities of protein available. This paper describes a method for the production of ILTV-encoded gpG in a stirred tank, expressed in an insect cell line of Spodoptera frugiperda (Sf-9) after infection with baculovirus BV-gpG. For this purpose, it was necessary a previous characterization of Sf-9 cells growth and the optimization of gpG production on a small scale. After that, we defined operational parameters in the Biostat B plus bioreactor to ensure the optimal hydrodynamic environment in order to guarantee cells growth and infection.

The cellular growth of Sf-9 was characterized, registering a specific growth rate (μmax) of 0.026/h, a population doubling time (PDT) of 27 hours and a maximum cell density of 8.7x106 cel/mL. Shake flask studies such as multiplicity of infection (MOI), harvest time, time of infection and density of infection were conducted to assess conditions to be used in a bioreactor. GpG production was higher when the cells were harvested at 72 hpi and independent of the multiplicity of infection (MOI) used in the range from 1.2 to 20. The Sf-9 cells that were infected in an early exponential phase showed a greater production than cells infected in late exponential phase. The optimal peak cell density at infection varied depending on the use of conditioned medium (2x106 cells/mL) or fresh medium (7x106cells/mL). On the bioreactor, was observed that using at agitator speed of 170 rpm, at aeration rate of 0.04 vvm and at a pO2 of 40%, the Cellular integrity was no affected by a shear stresses of 0.38 N/m2 with a Reynolds number of 20x104 in sparging condition. At 3,5 L bioreactor scale an efficient production was achieved by infecting the culture on exponential phase at a concentration of 2x106 cells/mL using a MOI of 2 pfu per cell and harvesting the cells 72 hpi. Using a ELISA sandwich, a volumetric yield of gpG was determined, it was 3.66 mg/L and it is 2-fold higher that yield of shaker flask. This data demonstrates the feasibility of gpG production by insect cells infecting with baculovirus on a bioreactor scale.

Introduction:

Bioreactors are the vessels/containers which give biological, biochemical, and biomechanical requirements for the optimal growth of the fermenting microorganisms and/or biochemical reactions on the economic scale for the synthesis of desired products. Efficient bioreactors are capable of maintaining the specified biological activity by controlling the temperature, pH, fluid velocity, shear stress, mass and warmth transfer, O2, CO2, and nutrient supply, reaction rate, and cell growth. Bioreactors are utilized in all domains of large-scale industrial biotechnology where an outsized scale production is required.

UASB may be a specialized bioreactor specially employed for wastewater treatment. It is also used for hydrogen production using different waste, both at lab-scale and pilot-scale. This bioreactor is effective in conversion of organic matter into hydrogen and maintenance of high cell concentration inside the reactor. Its major application is for sugar industry waste, food industry waste, beverage industry waste, and distillery industry waste treatment. Anaerobic granular sludge bed technology refers to a special quite reactor concept for the anaerobic treatment of wastewater at a high rate. This concept was first initiated with USAB reactor. The wastewaters pass upwards through an anaerobic sludge bed where the microorganisms within the sludge come and get in touch with wastewater substrates. Microorganisms present within the sludge bed can naturally form granules of 0.2–2 mm diameter and have a high sedimentation velocity and thus resists wash out from the system even at high hydraulic load. The resulting biogas production takes place under anaerobic degradation process. The released gas bubbles occupation upward motion can cause hydraulic turbulence and supply mixing within the reactor with none mechanical parts. When comparing with other anaerobic bioreactors, it contains granule sludge and internal three phase GSL device (Gas/sludge/liquid separator system).

The operating conditions of the UASB acid genic reactor, like concentration of solids within the feed, retention time, organic loading density, pH, and flow recirculation, were studied to maximise hydrogen production. In order to predict the steady-state performance of granule-based hydrogen-producing UASB, the reactor can be simulated using neural network and genetic algorithm and a model was designed, trained, and validated.

Biography:

Juana Quispe earned her Bachelor of Genetic and Biotechnology degree at the University of San Marcos in Lima-Peru. In her thesis work she evaluated the production of recombinant proteins using a Baculovirus System. Then, Juana worked at the FARVET company where she development new vaccines for avian species using a CRISPR-Cas9 system. Juana Quispe is currently a Master´s student of Biotechnology at the University of San Marcos. She is working in the laboratory of Dr Martha Valdivia where she applies different biotechnology tools to improve animal´s fertility.