**Introduction:** The production of alternate sources of fuels has been the area of immense research and has undergone a lot of development in the recent years. Once such fuel is biodiesel. Production of biodiesel occurs through transesterification reaction which also produces glycerol as a by-product. Even though the applications of glycerol are plenty, it has to be purified first. However, crude glycerol as it is with minimal purification steps can be used for the production of another source of alternative fuel i.e. bioethanol. *Bacillus Cereus* is one of the organisms that assimilates glycerol and uses it as carbon source for bioethanol production. Hence, the entire process serves as a dual-fuel system. Two reactors: Stirred tank batch bioreactor and Bubble Column bioreactor are fabricated to overcome the fermentation of viscous medium such as glycerol fermentation using conventional shake flask studies.

**Objectives:** The intention of this project was to scale-up and overcome the problems faced by the previous bioreactor model and to determine the effect of different purity stages of crude glycerol on bioethanol production. The objectives set are:

- To design and fabricate laboratory scale bioreactor for research/academic purposes and to determine the scale-up parameters
- To study the effect of crude glycerol purification stages on ethanol production
- To screen and scale up the fermentation process

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**Methodology:** *Bacillus cereus* (Eq. ATCC 14579T, facultative aerobic bacterium) isolated in the lab from honey was used as microbial source for fermentation. The glycerol was obtained from Biodiesel production unit producing biodiesel from Pongamia oil using NaOH as catalyst.

**Fabrication of Bioreactors:** Stirred tank batch bioreactor is fabricated with the following specifications: Total volume = 500ml; Working volume = 200ml to 350 ml; Aeration = 0.2 to 1.5 LPM; Agitation = 40 to 400 RPM; L/D ratio = 1 to 1.5; Reactor diameter = 70 mm; Agitator diameter = 50 mm; Ring type sparger.

Bubble column bioreactor has the following specifications: Total volume = 200ml; Working volume = 100 to 150 ml; Reactor height = 500mm; Reactor diameter = 25mm; Aeration rate = 0.2 to 1.5 LPM, Height of the liquid = 70 to 300 mm.

**Crude Glycerol Purification stages:** **Preliminary step:** The crude glycerol obtained from Udupi District Biodiesel Demonstration and Information center was initially heated at 80°C for 30min to evaporate all volatile matters that interfere with the fermentation. **Stage 1:** The crude glycerol then is treated with Ortho-Phosphoric acid until the pH reduces to 2. The resulting solution is kept in a separating flask for 12hours. 3 layers will be formed. Bottom layer being the salt layer is removed. The middle layer being the glycerol layer is collected then neutralized with NaOH. This becomes the stage-1 product. **Stage 2(a):** The stage 1 product is centrifuged at 9400 RPM for 15 min to further separate the fatty acids. The very thin layer at the top is removed using a syringe and the bottom layer of glycerol is used a stage 2(a) product. **Stage 2(b):** The stage 1 product is treated with Iso-propyl alcohol in the ratio (2:1::glycerol:IPA). It is mixed for 30min and left to stand still for 12hours. The top layer containing IPA and glycerol is removed and distilled to recover the IPA. The residue now becomes stage 2(b) product.


**Figure 1:** Stirred tank batch bioreactor

**Fermentation:** The operating parameters used here are the optimum conditions determined by previous work. Pure glycerol is used as the control fermentation. All fermentation is done in duplicates. Glycerol concentration used is 70g/L. The inoculum used is 2% 24 hour old culture of *B.cereus*. The fermentation is done for 48 hours with 0.5LPM aeration and 200RPM agitation. Glycerol was estimated using sodium periodate and ethanol was estimated using potassium dichromate method.
Results: Two types of bioreactors; stirred tank batch bioreactor of working volume 250ml and bubble column reactor of working volume 125ml were fabricated. The viscosity of the fermentation medium was found to be 1.3 times greater than that of water at 30°C and density at 30°C was 1065 kg/m³. For stage-1 product, ethanol yield in batch bioreactor was 0.142±0.003 and for stage-2(b) product, ethanol yield in batch bioreactor was 0.134±0.0008. It can be noted that, a simple purification step involving acidification of crude glycerol using OPA is sufficient for bioethanol production and yield maximum amount of ethanol. The mass transfer coefficients for the reactors were evaluated for scale up. Upon scaling to 1:6 volume ratio, the yield of ethanol reduced. The reactor was further scaled up to 1 lit production volume. In comparison, the yield reduced slightly compared to the previous work but the drastic reduction in purification requirements cannot be overlooked.