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Comparative Study of Yeast Growth Kinetics in Different Reactors

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Abstract: *This study aims at investigating three different reactors (air-lift fermenter, bubble column reactor, continuous stirred-tank reactor) to evaluate their performance with respect to baker's yeast (Active Dry Yeast powder) growth using YDM (Yeast Dextrose Media) in shake flask culture in the B.O.D incubator under optimum conditions. Conventional methodologies were implemented to investigate different parameters of yeast growth kinetics in the reactors viz., dry cell mass concentration, residual glucose concentration and cell count. It was finally estimated that among the three bioreactors, in the CSTR, running with an agitator speed of 650 rpm, the final dry cell mass concentration attained after 48 hours was 0.162gm/100 ml and almost all of the initial glucose (0.6 gm/100ml) was utilized within first 12 hours*

Keywords: *Airlift fermenter, agitator, bubble column reactor, baker's yeast, CSTR, turbidity (NTU).*

I. Introduction

Long before anyone understood the concept of bioreaction, humans took advantage of its results [4]. Bread, cheese, wine and beer were all made possible through what was traditionally known as fermentation— a little-understood process, successful more by chance than design [1, 2]. The fermentation process, being the precursor to modern bioreactions has been used since prehistoric days, with major advancements in the field of technology as well as biology. By definition, a bioreactor is a system in which a biological conversion is affected. Primarily the bioreactors referred to only mechanical vessels in which (a) organisms are cultivated in a controlled manner and/or (b) materials are converted or transformed via specific reactions [4]. Bioreactors differ from conventional chemical reactors in that they support and control biological entities. The bioreactor systems provide a higher degree of control over process upsets and contaminations, since the organisms are more sensitive and less stable than chemicals. [5] Organisms, influenced by their morphology and the bioreaction medium, are shear-sensitive to varying degrees. A number of bacteria, yeast and fungi cultures that can be relatively tolerant of high-shear environments exhibit a robustness in high-energy mixing vessels.[3] Mixing within the bioreactor is integral to efficient heat and mass transfer during the production phases, which places additional constraints on the suitable agitation mechanism and rheology of the bioreaction medium. In bioreactors, higher selectivity is of prime importance compared to rate [6, 7]. The effectiveness of the bioreactors depends on the suitable reactor parameters, including: controlled temperature, optimum pH, sufficient substrate, gas evolution [10, 11] etc.

1.1 Continuous Stirred tank reactor systems: The defining characteristic of continuous bioreaction is a perpetual feeding process [14]. A culture medium comprising of microorganisms is continuously fed into the bioreactor to maintain the steady state. The reaction variables and control parameters remain consistent, establishing a time-constant state within the reactor [15]. The result is continuous productivity and output.

1.2 Bubble Column reactor systems: Also known as a tower reactor, it has a bubble column containing a draught tube. Air is typically fed through a sparger ring into the bottom of a central draught tube that controls the circulation of air and the medium. [8, 9] Air flows up the tube, forming bubbles, and exhaust gas disengages at the top of the column. The degassed liquid then flows downward and the product is drained from the tank.

1.3 Airlift external-loop reactors

Another type of airlift system is the airlift external-loop reactor system, which is used primarily for batch operation. [13] This system consists of a riser and an external downcomer, which are connected at the bottom and the top, respectively. [12] As the injected air at the bottom of the riser creates gas bubbles that begin to rise through the main tank, exhaust gas disengages at the top and the resulting heavier solution descends through the downcomer.

II. Materials and methods

2.1 Experiments in batch growth:

The fermenters were sterilized by autoclaving at a temperature of 121°C for 15 minutes. The bulk medium was sterilized batch wise and then inoculated with a strain of baker's yeast. For batch operation the reactors were aseptically filled with inoculated medium with the help of a peristaltic pump. The culture volume in each of the reactor was 2-3 liters approximately. The sterilized air was introduced through a sparger (Sintered glass plate with 200 µm) and fermentation temperature in the reactors was maintained at 29 ±1°C.

All three reactors were operated under identical operating conditions. Samples were collected through the sampling port at an interval of one hour with the help of a peristaltic pump.

2.2 Materials

Baker's yeast (Active Dry Yeast powder) was selected for investigation. The composition of the YDM (Yeast Dextrose Media) is provided in the TABLE below.

Table 1: Composition of YDM

Media Constituents	Quantity
Dextrose	0.6%(6gm/100ml)
Yeast extracts	0.3gm/100ml
Peptone	0.5gm/100ml
NaCl	0.5gm
Distilled water	100ml
pH	4.5-5.0

2.2.1 Inoculum Preparation

The reagents were mixed in a conical flask of 250ml capacity, carefully cotton plugged and autoclaved for 30mins.The Active Dry Yeast globules were added to the media in a laminar hood aseptically, and placed in the shaker at 30°C for 48 hours.

2.2.2 Turbidity estimation

Composition of Standard NTU solution: 10ml of formazine mixed with 90ml of water, to prepare a standard solution of 400NTU.The set points of the turbidometer were measured with 20ml of water and 20ml of 400NTU standard solution respectively. 20ml of the aliquot samples were poured in the turbidometer tubes and the turbidity was measured.

2.2.3 Standard curve (NTU vs Dry cell mass concentration [gm /100ml]) Preparation

Two aliquots of the fermented broth of 25ml each were centrifuged at 2000 r.p.m for 5 mins. After centrifugation the supernatant was discarded and the pellet was kept aside. The pellet was washed with water two times and then 25ml of distilled water was added to it and to mix it well. From this stock solution five samples of net volume 20ml each were prepared via serial dilution method. From the prepared samples 15ml aliquots were taken out in aluminium foil tray and heated in hot air oven at 100°C for 1hr to obtain dry cell mass. The remaining 5ml of each sample were used for turbidity measurement of corresponding concentrations.

2.2.4 Glucose estimation

Glucose was estimated quantitatively by Miller method. To 0.5ml of the aliquot samples, 3ml of DNSA and 0.5 ml of distilled water was added, heated in a boiling water bath for 3 minutes, and was consequently cooled down to the room temperature. 21 ml water was added to make up, a total volume of 25ml. The optical density (O.D) was measured by colorimeter. With the help of a glucose standard curve, which was prepared by serial dilution method, unknown glucose concentration was determined from the known O.D value.

2.2.5 Determination of cell count using haemocytometer

The yeast cells were counted using a haemocytometer via Dye-Exclusion method. 10µl of cell suspension was mixed with crystal violet in 1:1 ratio. The dye stained cell suspension was pipetted into the grooves of the haemocytometer and covered with a cover slip. The cells were observed under a compound

microscope at 10X and 40X respectively.

III. Results and Discussions

All the three bioreactor models fit closely to the experimental data and resembled a similar pattern of growth in their respective dry cell mass concentration, which in turn resulted in a considerable depreciation in the residual glucose concentration in each of the three reactors.

In accordance with yeast growth kinetics there should have been a significant lag phase for the initial half hour, which is not evident in the present study, as the relative data was not recorded under the operating lab conditions for the respective bioreactor models.

3.1 Estimation of glucose concentration vs time:

The overall residual glucose consumption in all the three bioreactors showed quite similar pattern of depreciation .In case of the airlift fermenter (Fig .1),the residual glucose concentration depreciated from 0.41 gm/100ml to almost zero within a stretch of 48 hours, comprising of a steep decrease in the concentration profile for the initial 7 hours. In Bubble column (Fig .2), the residual glucose concentration decreased from 0.51 gm/100ml to ultimately zero over a stretch of 48 hours. Bearing resemblance to the above two models, even the glucose concentration in CSTR (Fig .3), decreased from 0.6 gm /100ml to finally zero, gradually over a period of 48 hours.

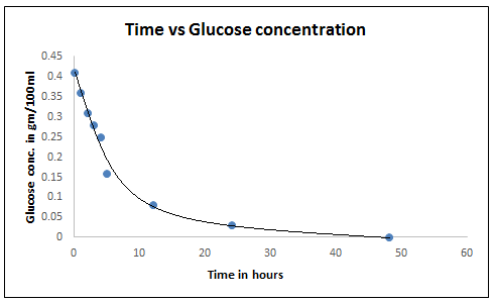

Figure.1 Trends of glucose consumption over time in Airlift fermenter

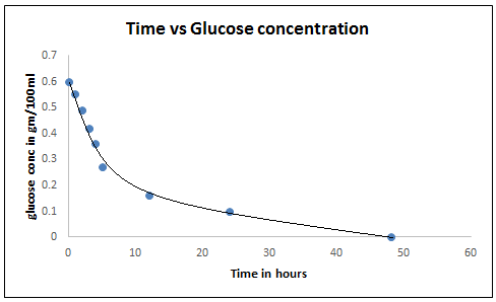

Figure 2. Trends of glucose consumption over time in Bubble column reactor

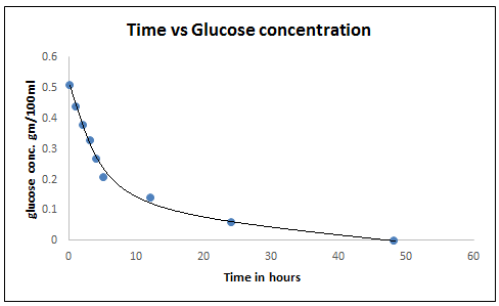

Figure 3. Trends of glucose consumptionover time in CSTR.

3.2 Estimation of alcohol percentage

In airlift fermenter 175ml of distillate obtained from 2 liters of fermented media produced 0.18% of alcohol. Whereas in case of CSTR, 32 ml of distillate was obtained from 500 ml of fermented media, which approximately produced 0.067% of alcohol.

3.3 Estimation of dry cell mass vs time

The airlift fermenter (Fig .4) reported a considerable increase in the dry cell mass concentration from an initial value of 0.035 gm/100ml to 0.134 gm /100 ml over a stretch of 6 hours. Eventually the cell growth decelerated but the cell mass increased to 0.148 gm /100ml over 24 hours, ultimately reaching the stationary phase in the next 24 hours .The initial dry cell mass concentration of 0.01 gm/100ml rose to 0.047 gm /100 ml over 6 hours, in the bubble column reactor(Fig .5). Further trending with the growth kinetics pattern the cell mass increased to 0.56 gm/100ml in 24 hours and ultimately reached the stationary phase in 48 hours. The CSTR (Fig .6) depicted a steep rise in the dry cell mass concentration for the initial 7 hours from a value of 0.03 gm /100 ml to 0.147 gm /100 ml and a stationary phase was observed from 24 hours up to 48 hours with a final concentration of 0.162 gm/100 ml.

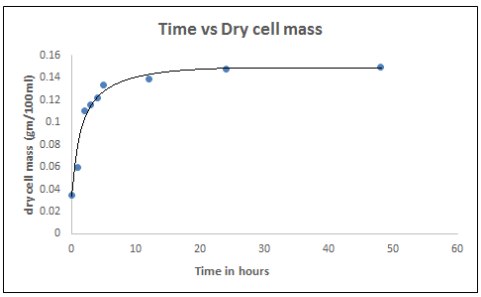

Figure 4. Trends of dry cell mass concentration over time in Airlift fermenter

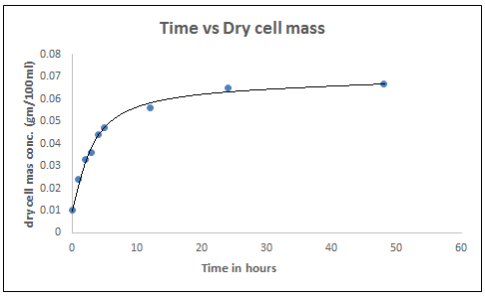

Figure 5. Trends of dry cell mass concentration over time in Bubble column reactor

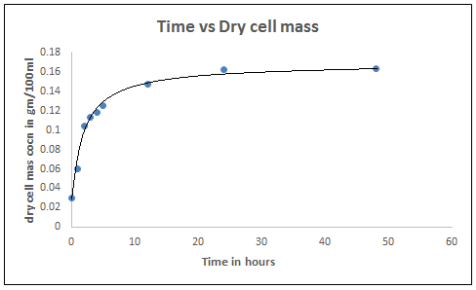

Figure 6. Trends of dry cell mass concentration over time in CSTR

3.4 Estimation of Cell count

The graphical plot (Fig .7) represents an exponential increment in the number of cells counted for the initial 24 hour time span in the airlift fermenter. From 24 hour onwards, gradually the trend achieves a saturation point.

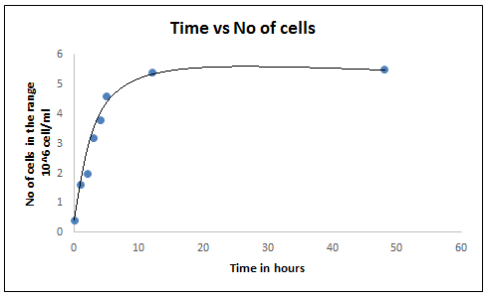

Figure 7. Trends of cell count over constant time in Airlift fermenter

IV. Conclusion

The maximum amount of growth was observed in case of CSTR, with a final dry cell mass concentration of 0.162 gm / 100 ml after 48 hours. Nearly all of the glucose was utilized within first 24hours. Due to continuous mechanical stirring system, finer bubbles were produced, which aided in a greater mass transfer in CSTR compared to other reactors. Also the constancy of continuous bioreaction provides a more accurate picture of kinetic constants, maintenance energy and true growth yields. The growth rates can be regulated and maintained for extended periods. By varying the dilution rate, biomass concentration can be controlled and secondary metabolite production can be sustained simultaneously along with growth. Hence CSTR provided a good condition for the baker's yeast cells to grow.

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