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WORKING OF A BIOREACTOR: A MINI REVIEW

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ABSTRACT

This mini review is directed towards accumulating advances in bioreactor and bioprocess design in growing microorganism for various purposes. The state-of-the art of bioreactor configuration and operation is discussed. Briefly, four different bioreactor operation techniques i.e. batch cultivation, continuous culture enhancement; fed-batch cultivation and continuous culture with complete cell recycle technique have been discussed. Limitations and constraints of these operation techniques are also emphasized.

KEYWORDS: Bioreactor, Bioprocess, Microorganisms**,** fed-batch cultivation, batch cultivation, continuous culture

INTRODUCTION

A bioreactor is a device that is engineered to grow microorganisms in an environment suitable for its growth. It consists of a large vessel, made of steel or glass, where in a microbe grows and helps in production of different products. A bioreactor can be operated to grow microbes, fungus, algae, plants, cells or tissues. The twenty first century is the age of bioreactors. Progress in bioscience and biotechnology has lead to the development of many bio-related industries. Commercial production of products produced by microorganisms requires two distinct bodies of knowledge- molecular biology and process engineering. Molecular biology enables expression of genes from plants, microorganisms, or animal origin that can be used for industrial production *(Stoger, 2012; Mitra, 2011)*; while background in process engineering empowers us to design and operate large-scale plants for growing and subsequent purification and formation of products *(Roosta, 2012)*. In the early days it was thought that scale-up of reactor was simply a matter of using larger volumes, i.e. the condition merely necessary was to use a larger fermentor vessel with a larger culture media volume. Such an approach results not only in product variability, both in terms of product and quality, but also expensive operating costs. Hence, a systematic study of process engineering principles is needed for scaling up and operation of biotechnological processes for manufacturing various products. Development of new biological products and tighter regulations on product quality, stability and consistency of a bioprocess are becoming very important. To cope-up with these requirements, a quantitative knowledge of culture dynamics and stability is needed, which helps in understanding, optimizing and controlling of a process. Since late sixties, dynamic behavior of microbes was the objective of a number of theoretical workers *(Ramkrishna,1967; Sokól, 1988; Luong, 1987)*. Thorough and comprehensive experimental studies have been reported only recently *(Xudong, 2003; Krzeminski, 2012; Upadhyaya, 2012; Achilli, 2011)*.

To facilitate the production of a variety of products, the possession of a large amount of enzyme is crucial. The use of shake flasks in small scale production is common as the need to satisfy various external conditions is non-existent. With respect to large scale application, however, not only does the need exist, but also the means by which we accomplish our goals changes accordingly from shake flask to bioreactors. For instance stirred-tank bioreactors have found a place in the enzymatic hydrolysis of biomass to fermentable sugars *(Riedlberger, 2012)*. Alginate immobilized *Lactobacillus fermentum* CGMCC2921 cells have been used in production of D: -tagatose, a functional sweetener in foods, beverages, and dietary supplements *(Xu, 2011)*. Strains of the robust *Saccharomyces cerevisiae* are used in industries for production of ethanol. A biocatalyst for production of ethanol from this xylose in a continuous bioreactor has also been achieved *(Silva, 2012)*. An enzymatic reactor with lipase immobilized on a monolithic polymer support been shown to catalyze the transesterification of triacylglycerides into the fatty acid methyl esters that are commonly used for biodiesel *(Urban, 2012)*. Bioconversion of wastes from olive oil industries by vermicomposting process i.e. bioconversion of wet olive cake by low-cost biostabilization, using the epigeic earthworm Eisenia Andrei is another interesting application where in a bioreactor was used for use both in conventional and organic agriculture and soil-restoration programs *(Melgar, 2009)*.

1. Construction and Working of a Bioreactor:

The heart of a bioprocess used for manufacture of biological organisms, is a bioreactor. Figure 1 shows a typical vessel of a running bioreactor. Generally speaking, a bioreactor usually consists of a large vessel, which ranges from few to 100,000 liters, and is made of a glass or stainless steel, and is equipped with a temperature, pH and dissolved oxygen (DO) measurement probe and control system. This main vessel, containing the culture media is surrounded by a double jacketed layer. Media filled in the vessel is sterilized by autoclaved at 121ºC for 30mins. Warm water adjusted to a specific temperature passes through the space between the double jacketed layer and maintains the temperature of the system according to the requirement. The bioreactor is equipped with an agitation system to keep the contents uniformly mixed and provide consistent oxygen transfer. Continuous oxygen is supplied via an air pump, which passes through a cylindrical glass tube filled with silica gel. An agitator, attached to many baffles, helps in mixing the culture contents and allows uniform air and nutrient supply to all the microorganisms. pH, temperature and dissolved oxygen are simultaneously

controlled, monitored and collected. For sample analysis like enzyme activity, glucose level, viable cell count etc a sampling outlet is used, which is generally located on the reactor lid, via a peristaltic pump. Generally, large-scale microbial cultivation or cell culture, and purification steps are carried out in a step-wise manner.

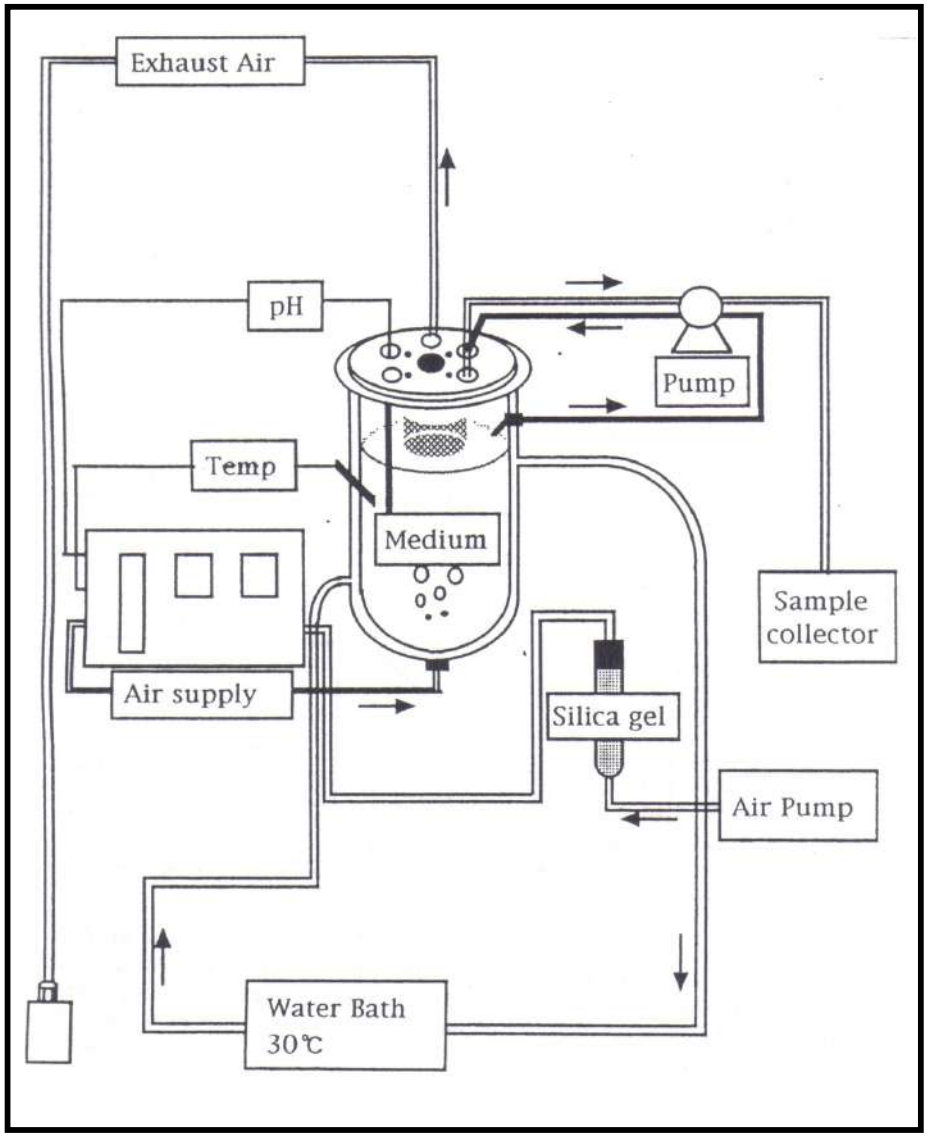

Fig. 1 – Schematic diagram of a typical Bioreactor

2. Types of Bioreactors

Bioreactor can be constructed to patronize numerous purposes. Not only can mass production of an enzyme be procured, but immobilization of the enzyme in the bioreactor to affect hydrolysis of oils can also be facilitated *(Liang, 2012; Anuar, 2011; Chen, 2010)*. Some of the commonly used supports are: silica gel *(Alvarez, 2009; Karagulyan, 2008; Wei, 2001)*, hydrophobic supports *(Al-Duri, 1995; Brady, 1988)*, anion exchange resin *(Sonnet, 1994)*, polymeric membranes *(Rucka, 1990)*, polyaziridines *(Wood, 1990)*, biomass support particles *(Nakashima, 1988)* and many others. Oil hydrolysis can also be accomplished in the bioreactor, without immobilizing the enzyme. Thus, a bioreactor that is suitable for practical use, and has the capability to conduct various enzymatic reactions, separation of products and recovery of the enzyme, must be developed. Li et al. *(2012)* have constructed a fixed-bed biofilm system designed to remove perchlorate from drinking water, linking bioreactor operation and performance and microbial community structure. Interesting work on membrane bioreactors where hemoglobin was isolated from bovine slaughterhouse erythrocytes has been designed at a pilot scale *(Stojanović, 2012)*. Timmins et al. *(2012)* used closed system to isolate and scale human placental mesenchymal stem cells and subsequent cultivation on microcarriers in scalable single-use bioreactor system. This is a novel design as industrial manufacture of mesenchymal stem cells are currently performed in an open process or stirred bioreactor systems using microcarriers but it has turned out to be extremely labor intensive, costly and impractical for a small number of patients. There is an intriguing review on stem cell cultivation in bioreactor *(Rodrigues, 2011)*. Construction of draft tube type reactor by Tanigaki et al. *(1995)* to achieve both enzymatic reaction and separation of oil and water is another interesting design. Oils can also be hydrolyzed by adding it in the culture media itself. Somrutai et al. *(1996)* have shown that as growth of the microorganism starts enzyme production is facilitated, this in turn succors in hydrolysis of the oil. However, as expected batch-wise cell-free translation in a bioreactor is not popular industrially due to its low productivity *(Yamamoto, 1996)*. van der Padt et al. *(1992)* have chemically synthesized monoglycerides by constructing a 'membrane bioreactor with an in-line adsorption column'. Although high temperature was required, they have shown that this kind of reactor can be successfully constructed. Hydrolysis or acidolysis of oil using enzymes in transfer cell and super critical carbon dioxide, respectively, have also been studied *(Kawano, 1994; Gunnlaugsdottir, 1995; Bhatia, 1999)*.

3. Bioreactor operation techniques

Operation of a reactor varies according to the nature of its product requirement. The operation technique also relies on the type of microorganism, on the enzyme produced, toxicity of product, reaction condition, substrate inhibition and many other factors. This section amply demonstrates the unique possibility of different culture techniques in a bioreactor. In general, the reactor can be operated in four different ways namely: batch, continuous culture enhancement, fed-batch and continuous culture with complete cell recycle technique. These techniques are briefly discussed.

3.1 Batch cultivation technique (BCT)

Among the various strategies available for selection, the classical batch type enrichment technique holds a special place. The principal reasons for its popularity are its lucidity and wide range of selective growth that can be applied. In the past, strong interest in organisms selected in batch cultures overshadowed the fact that enrichment techniques for organisms lacking pronounced metabolic specificity were practically non-existent. BCT has been used by many scientists to conduct standardization of mass enzyme production *(Ishihara, 1989; Tan, 1985; Anu Appaiah, 1995)*. The reactor can be run at different air-flow rates *(Sharon, 1999)* to attain maximal enzyme activity and viable cell count. In *1989* Kreis and Reinhard constructed special bioreactor systems, such as airlift or drum-type fermentors which were devised for the mass cultivation of plant cells. In those days biological rather

than technological problems were the main obstacles to more common use of fermentor-cultured plant cells in industry. Batch cultures were also used to grow somatic embryos of *Digitalis lanata* st. VII in gaslift fermenters *(Greidziak, 1990)*. Studies on the stability of heterotrophic green micro-algal strain *Prototheca zopfii* RND16 immobilized in polyurethane foam cubes during degradation of mixed hydrocarbon substrate, which was composed of n-alkanes and polycyclic aromatic hydrocarbons in five successive cycles of repeated batch cultivation was reported *(Ueno, 2008)*. A study on on-line monitoring of the growth of *Escherichia coli* in batch cultures by bioluminescence was performed using a photodiode, a photodetector amplifier and a recorder. The study indicated that even though the actual metabolism of cells in a culture is at a very low level or completely shut-down, the cells retain their capability to be culturable. This on-line technology has a number of potential uses in the laboratory and industry *(Marincs, 2000)*. There is no doubt that batch cultivation technique was a landmark in the history of microbiology as it revealed various microorganisms and their significance in nature. Indeed, the reproducibility of batch enrichments generally increases when the initial conditions are made more extreme.

3.2continuous culture enhancement technique (CCET):

Continuous culture of microorganisms is a technique of umpteen importance in biotechnology. A continuous culture system consists of a culture vessel with growing microorganism, which is continuously supplied with fresh nutrition (Fig 2). The inflowing medium is instantly mixed with the culture, thus ensuring homogeneity of the system. At the same time the volume of the culture is kept constant by an overflow system. Dilution rate (D) is calculated using the formula: **D (h^{-1}) = f / V;** where D is dilution rate (h^{-1}), f is flow rate of fresh feed (L/h) and V is total volume of culture in the bioreactor (L). CCET offers the possibility of selecting microorganisms in a constant environment on the basis of their different growth rates at different substrate concentrations, as it is possible to maintain a steady state concentration of the 'growth-limiting nutrient' in the culture, which permits growth of the microorganism at sub-maximal rates. Furthermore, in CCET parameters such as pH, oxygen tension, concentration of products and population densities can be easily controlled. The ability to obtain and maintain over a long period of time, a desired set of environment conditions in the bioreactor, and the 'open' nature of the CCET enables one to screen a large number of microorganisms rapidly with a view to selecting one better adapted to these conditions *(Gilbert, 1991; Becker, 1998; Vidmar,1984)*. There are different applications where CCET can be employed. Production of gram quantities of monoclonal antibodies directed against human fibronectin were cultured in VITAFIBER II and VITAFIBER V hollow fiber bioreactors using defined, serum-free WRC 935 medium using continuous feed hollow fiber bioreactor cell culture systems *(Heifetz, 1989)*. A comparison on perfusion to batch systems showed improve in the quality of the product in the conditioned media in terms of biological activity and structural integrity in biopharmaceuticals *(Prior, 1998)*. Relating molecular and structural properties of in vitro reconstructed cardiac muscle with its electrophysiological function using an in vitro model system based on neonatal rat cardiac myocytes, three-dimensional polymeric scaffolds, and bioreactors was another fascinating application of bioreactor in cardiac tissue engineering *(Papadaki, 2001)*. Liquid-solid fluidized bed reactor has been shown to carry out sulfate reduction with a mixed culture of sulfate reducing bacteria, where the bacteria were immobilized on porous glass beads. Low specific gravity of these hydrated beads allows operation at low liquid recirculation rates *(Nagpal, 2000)*. The continuous culture of Clostridium thermocellum, a thermophilic bacterium was shown to be capable of producing ethanol from cellulosic material *(Bothun, 2004)*. We have also shown continuous production of active Pseudomonas aeruginosa KKA-5 lipase in a bioreactor. This lipase was further purified to hydrolyze castor oil *(Sharon, 1999)*. The application of NIR in-line to monitor and control fermentation processes was investigated by Tosi et al.*(2005)*, where a fiber optic probe immersed into the culture broth and connected to a Near-infrared spectroscopy instrument was used in determination of biomass, glucose, and lactic and acetic acids during fermentations of *Staphylococcus xylosus* ES13. A circulating packed bed bioreactor and a culture was developed by Huang et al. *(2012)* to study batch and continuous biodegradation of trans-4-methyl-cyclohexane carboxylic acid (trans-4MCHCA), a mixture of cis- and trans-4-methyl-cyclohexane acetic acid (4-MCHAA), and mixture of these three naphthenic acids, which pose as a serious environmental concern associated with processing of the oil sands. Continuous flow bioreactors coupled with bicarbonate or nonbicarbonate buffer systems supplemented media for articular cartilage tissue engineering has been successfully constructed for articular cartilage tissue engineering also *(Khan, 2011)*. Manure waste from dairy farms is used for

methane production. Importance of mixing and its critical role in design of large scale anaerobic digesters is been described in a 100-L digester *(Borole, 2006)*. Microbial diversity of anammox bacteria community enriched from different types of seed sludge in an anaerobic continuous-feeding cultivation reactor was reported in 2009 *(Date, 2009)*. A Continuous-flow biofilm reactor was used to show chloroform cometabolism by Rhodococcus aetherovorans BCP1, a butane-grown aerobic pure culture *(Ciavarelli, 2011)*. CCET has also been applied to the clinical areas where fluidized bed bioartificial liver was proposed as a temporary support to bridge patients suffering from acute liver failure to transplantation. In such a bioreactor, alginate beads hosting hepatocytes are in continuous motion during at least six hours without altering the beads significantly. Such a device could be used as a bioartificial organ in future *(David, 2006)*. In another study a semi-continuous four-channel colon simulator was used to study the effects of lactose on the growth and fermentation dynamics of colonic microbiota *(Mäkivuokko, 2006)*.An interesting review on fundamental technologies required for microchip-based bioreactors utilizing living mammalian cells and pressure driven flow was published in 2010 *(Sato, 2010*).

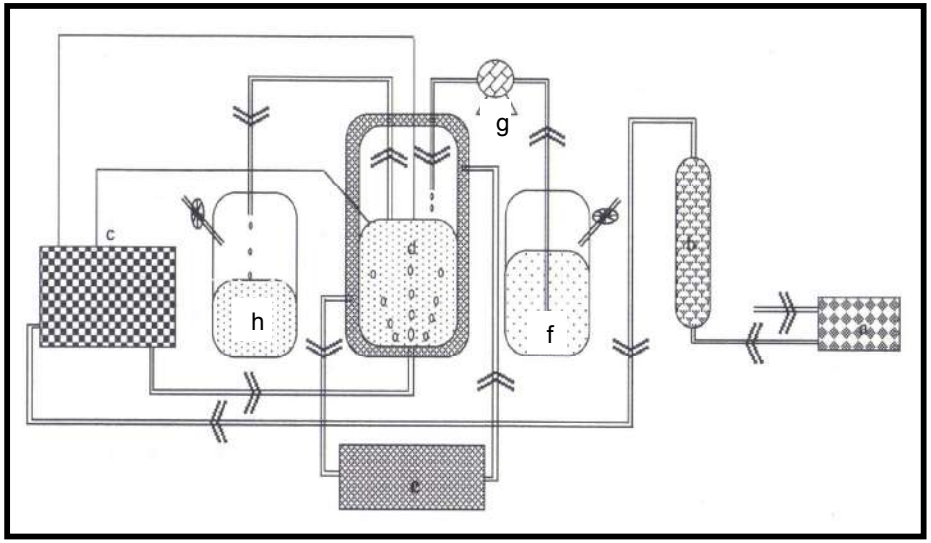

Figure 2. Schematic representation of a *Continuous Cell Culture System* (a- air pump, b-silica gel, c- pH, temperature and air flow rate detector, d- double jacketed reactor, e- water bath, f- autoclaved disposable tank containing distilled water in which air from the reactor flows out, g- peristaltic pump, h- sample injector)

3.2 Fed-batch cultivation technique (FBCT):

Fed-batch cultivation is commonly called 'Semi-batch cultivation'. FBCT is a proficient scheme to achieve high productivity of biomass and metabolites. There is an increasing demand for recombinant therapeutic proteins which constantly needs us to improve the efficiency and yield of these

biopharmaceutical products from microbes or mammalian cells. This is achievable only through proper understanding of cellular functioning. With the merit that concentrations of nutrients can be controlled favorably, fed-batch operations can be employed in many microbial industries. During growth the environment of microorganisms alters continuously. If conditions remain favorable growth continues until one of the essential substrates in the medium is depleted. If all the nutrients are in excess, then this substrate is called the 'growth-limiting substrate'. With depletion of the 'growth-limiting substrate', microbial growth decreases. The growth finally becomes negligible if additional 'growth-limiting substrate' is not supplied to the culture *(Bibal, 1998; Chartrain,1993*). Automatic feeding system was achieved by supplying oil in semi-batch culture along with continuous by monitoring of cell concentration with a laser turbidimeter, microcomputer and a pulse motor *(Ishihara, 1989)*. Enhanced lipase production from *Candida rugosa* and *Pseudomonas aeruginosa* was successfully obtained by using different control fed-batch operational strategies *(Gordillo, 1998; Jaeger, 1996)*. Continuous sucrose feeding (5 g/L h) to the immobilized-cell culture in an airlift bioreactor showed an increased production of enzymatic activity by about 107% compared with ordinary batch operation *(El-Sayed, 1990)*. *Pichia methanolica* expresses the human transferrin N-lobe protein. Various methanol concentrations controlled by an on-line monitoring and control system were investigated in mixed glucose/methanol fed-batch cultures, where maximum recombinant gene expression was observed at 0.7% (v/v) methanol concentration, resulting in maximum volumetric production of 450 mg of transferrin per liter after 72 h of elapsed fermentation time *(Mayson, 2003)*. Fed-batch fermentations of glucose by *Propionibacterium acidipropionici* ATCC 4875 in free-cell suspension culture and immobilized in a fibrous-bed bioreactor attributed not only to high viable cell density but also to favorable mutations that resulted from adaptation by cell immobilization in the fed-batch fermentor *(Suwannakham, 2005)*. A novel method for on-line monitoring and fed-batch control based on fluorescence measurements was developed by Hantelmann et al. *(2005)* In industrial fed-batch cultivations it is important to control substrate concentrations at a low level in order to prevent the production of overflow metabolites and thus optimize the biomass yield. Via in situ measurements and multivariate data analysis a chemometric model was established which rapidly detects ethanol production at aerobic *Saccharomyces cerevisiae* fed-batch cultivations. A new cultivation strategy was established that combined the advantages of temperature-limited fed-batch and probing feeding control in a 3 liter bioreactor *(de Maré, 2005)*. Batch-wise sorbitol addition as a co-substrate at the induction phase of methanol fed-batch fermentation by *Pichia pastoris* proved as a beneficial recombinant protein production strategy owing to ease and efficiency of the reactor *(Celik, 2009)*. A recent article was published on characterization and feasibility of a miniaturized stirred tank bioreactor (25ml) in terms of its power input, hydrodynamics, and volumetric oxygen transfer coefficient, to culture high density of *E. coli* in fed-batch fermentations *(Ali, 2011)*. Although FBCT is comparatively easily achieved in microbial cultures, insect culture application is still hampered by low cell densities in batch fermentations and expensive culture media. Marteijn et al. *(2003)* showed that with respect to the culture method, the fed-batch culture mode is often found to give the best yields. Recently Youn et al. *(2010)* used fed-batch cultivation techniques to study cultures of *Hansenula polymorpha* in order to develop an efficient biosystem to produce recombinant human serum albumin. A simple and effective fed-batch fermentation method was developed by Li et al. *(2010)* for production of Gamma-aminobutyric acid, a major inhibitory neurotransmitter in mammalian brains. An interesting report on fault detection and diagnosis in flexible process monitoring method was applied to industrial pilot plant cell culture data, which stated that this data from a relatively small number of batches (approximately 20) could be used to monitor for a wide range of process faults *(Gunther, 2007)*. Development of strategies for genetically engineering CHO cells to counter apoptotic death in batch and fed-batch cultures was accomplished by cloning caspase-2, -8 and -9 from a Chinese hamster ovary cDNA library and expressing it in *Escherichia coli*. Interestingly it was observed that specific inhibition of caspase-8 and -9 in CHO cells enhanced cell viability in batch and fed-batch cultures *(Yun, 2007)*. Fed-batch cultivation technique has also been used to process scale-up from 3-L to 2,500-L for production of monoclonal antibody from cell culture. The 2,500 L, fed-batch production process for Epratuzumab met

all scheduled batch releases, antibody quality was consistent and reproducible, and met all specifications, thus confirming the robustness of this process *(Yang, 2007)*.

3.3 Continuous culture with complete cell recycle technique (C4RT):

C4RT is a closed system with respect to biomass (solid) whilst open with respect to substrate and metabolites (liquid). Substrates added are immediately used up by the microorganisms thus keeping the free concentration low. As fresh substrate is added and being used up continuously, and external loop is required which could separate the biomass from the substrate. In this process, spend media and end products are removed from the system while the biomass is retained and re-circulated in the system. This system can grow microorganisms on toxic substrates as it avoids dubiety such as substrate and product inhibition. In this system it would be recommended to add fresh substrate at constant rate or increasing dilution rate. Adding substrate at constant rate could lead to cessation of cell growth as rate of substrate flow becomes insufficient to support further growth of the cells in the fermentor. However at high substrate flow rate, nutrient demand is met and there is increase in biomass and productivity until some other factor becomes limiting *(Berg, 1989)*. To overcome problems associated with substrate and product inhibition, and when high cell densities are required C4RT is employed *(Manelius, 1997; Borch, 1991).* C4RT is proved useful for cultivating mesophiles on toxic substrates. C4RT has also been reported in growth of thermophiles *(Nipkow, 1989; San, 1994)*

4. Concluding remarks:

Being a vast topic I have tried to cover as many topics as possible. Various cultivation techniques, their advantages, disadvantages and limitations are discussed that has lead to believe that a hard-and-fast rule cannot be postulated for explicit growth conditions for living and survival of a microorganism, as they differ from cell to cell. Knowledge about the existence of a bacterium, its favorable conditions for enzyme production or a specific product or a particular condition to carry out some reactions can be accomplished using these techniques. Executing these techniques and enzyme or any required product can be procured in large scale too. As enzymes are of potent importance in various industries, bioreactors proposes to be highly economical in its production. There are many novel publications in this area. I have tried my best to sum-up from many of the research in this short mini review. There is a lot more work being done and lots more that we still need to accomplish.

REFERENCES

1. Ali S., Perez-Pardo M.A., Aucamp J.P., Craig A., Bracewell D.G., Baganz F. (2011). '*Biotechnol Prog*. 2011 Aug 25. doi: 10.1002/btpr.708.
2. Achilli A., Marchand E.A. and Childress A.E., *Water Sci Technol*. 2011, 63 (12),2999
3. Anuar S.T., Villegas C., Mugo S.M., Curtis J.M., *Lipids*, 46 (6), 545
4. Anu Appaiah K.A, Karanth N.G.K., *Letts. Appl. Microb.*1995. 21 (1),65
5. Alvarez G.S., Foglia M.L., Copello G.J., Desimone M.F., Diaz L.E. *Appl Microbiol Biotechnol*, 2009,82(4), 639
6. Al-Duri B., Robinson E., McNerlan S., Bailie P., *JAOCS,* 1995, 72(12), 1351
7. Brady C., Metcalfe L., Slaboszewski D. ,Frank D., *JAOCS*, 1988, 65(6), 917
8. Bhatia S., Naidu A.D., Kamaruddin A.H., *Artif Cells Blood Substit Immobil Biotechnol*. 1999, 27(5-6), 435
9. Becker P., Abu-Reesh I., Markossian S., Antranikia G., Märkl H., *Appl Microb Biotech.* 1998, 48(2):184
10. Bothun G.D., Knutson B.L., Berberich J.A., Strobel H.J., Nokes S.E., *Appl Microbiol Biotechnol*. 2004, 65(2), 149
11. Borole A.P., Klasson K.T., Ridenour W., Holland J., Karim K., Al-Dahhan M.H., *Appl Biochem Biotechnol*. 2006, 129-132,887
12. Bibal B., Goma G., Vayssier Y., Pareilleux A., *Appl. Microb Biotech*.1988, 28(4):345
13. Borch E., Berg A.C., Holst O., J Appl Bact. 1991, 71, 2650269
14. Berg A.C., Holst O., Mattiasson B., *Appl Microb Biotech*. 1989, 30, 1
15. Chen J.P., Lin G.H., Appl Biochem Biotechnol. 2010,161(1-8),181
16. Chartrain M., Marcin C., Katz L., Salmon P., Brix T., Buckland B., Greasham R. J Ferment Bioeng.1993, 76(6),487
17. Ciavarelli R., Cappelletti M., Fedi S., Pinelli D., Frascari D. , *Bioprocess Biosyst Eng*. 2011 Nov 1. [Epub ahead of print]
18. Celik E., Calik P., Oliver S.G., Yeast 2009, 26(9),473
19. de Maré L., Velut S., Ledung E., Cimander C., Norrman B., Karlsson E.N., Holst O., Hagander P., *Biotechnol Lett.* 2005, 27(14), 983.
20. Date Y., Isaka K., Ikuta H., Sumino T., Kaneko N., Yoshie S., Tsuneda S., Inamori Y., J Biosci Bioeng, 2009 107(3), 281.
21. David B., Barbe L., Barthès-Biesel D., Legallais C., *Int J Artif Organs*.2006, 29(8),756
22. El-Sayed A.H., Mahmoud W.M., Coughlin R.W., *Biotechnol Bioeng*. 1990, 36(4), 338
23. Greidziak N. Diettrich B., Luckner M., *Planta Med*. 1990, 56(2), 175
24. Gilbert E.J., Drozd J.W., Jones C.W., *J Gen Microbiol*. 1991, 137(9),2215
25. Gunnlaugsdottir H., Sivik B. *JAOCS.*, 1995, 72(4),399

26. Gordillo M.A., Sanz A., Sánchez A., Valero F., MontesinosJ.L., Lafuente J., Solà C., *Biotechnol Bioeng.* 1998, 60(2): 156-168
27. Gunther J.C., Conner J.S., Seborg D.E., *Biotechnol Prog.* 2007, 23(4), 851
28. Heifetz A.H., Braatz J.A., Wolfe R.A., Barry R.M., Miller D.A., Solomon B.A., *Biotechniques.*1989, 7(2),192
29. Huang J., Nemati M,. Hill G., Headley J., *J Hazard Mater*. 201,2 30; 201-202:.
30. Hantelmann K., Kollecker M., Hüll D., Hitzmann B., Scheper T., J Biotechnol. 2005, 121(3), 410
31. Ishihara K., Suzuki T., Yamane T., Shimizu S., *Appl. Microb Biotech*. 1989, 31(1), 45
32. Jaeger K.E., Liebeton K., Zonta A., Schimossek K., Reetz M.T., Appl Microb Biotech. 1996, 46(2), 99
33. Krzeminski P., van der Graaf J.H., van Lier J.B., *Water Sci Technol*. 1012, 65(2), 380
34. Karagulyan H.K., Gasparyan V.K., Decker S.R., Appl Biochem Biotechnol. 2008, 146(1-3), 39
35. Kawano Y., Kawasaki M., Shiomori K., Baba Y., Hano T., J Ferment Bioeng. 1994, 77(3), 283
36. Kreis W., Reinhard E., *Planta Med.* 1989, 55(5), 409
37. Khan A.A., Surrao D.C., Tissue Eng Part C Methods. 2011 Dec 22.
38. The Importance of Bicarbonate and Nonbicarbonate Buffer Systems in Batch and Continuous Flow Bioreactors for Articular Cartilage Tissue Engineering. Luong J.H.T.. Biotech Bioeng. 1987, 29(2), 242
39. Liang Z.X., Li L., Li S., Cai Y.H., Yang S.T., Wang J.F., *Bioprocess Biosyst Eng.* 2012, Jan 7. [Epub ahead of print]
40. Li X., Yuen W., Morgenroth E., Raskin L., *Appl Microbiol Biotechnol*. 2012 Jan 19. [Epub ahead of print]
41. Li H., Qiu T., Huang G., Cao Y., *Microb Cell Fact.* 2010, 12(9), 85.
42. Mäkivuokko H.A., Saarinen M.T., Ouwehand A.C., Rautonen N.E., *Biosci Biotechnol Biochem*. 2006, 70(9), 2056
43. Mayson B.E., Kilburn D.G., Zamost B.L., Raymond C.K., Lesnicki G.J., *Biotechnol Bioeng* 2003,. 81(3):291
44. Marteijn R.C., Jurrius O., Dhont J., de Gooijer C.D., Tramper J., Martens D.E., *Biotechnol Bioeng*. 2003, 81(3), 269
45. Magdalena Rucka, Bożena Turkiewicz , Janusz S. Żuk. JAOCS.1990, 67(12), 887
46. Melgar R., Benitez E., Nogales R. *J Environ Sci Health* B.2009, 44(5), 488
47. Mitra D., Rasmussen M.L., Chand P., Chintareddy V.R., Yao L., Grewell D., Verkade J.G., Wang T. et al. *Bioresour Technol.* 2011. Dec 14.
48. Marincs F. *Appl Microbiol Biotechnol*. 2000, 53(5),536
49. Manelius Å., O. Holst *Letters in Applied Microbiology* 1997, 24, 384
50. Nipkow A., Zeikus J.G., Gerhardt P., Biotechnol Bioeng. 1989, 34(8),1075.

51. Nagpal S., Chuichulcherm S., Peeva L., Livingston A. , *Biotechnol Bioeng*. 2000, 70(4), 370
52. Nakashima T., Fukuda H., Kyotani S., Morikawa H. *J Ferment Technol.* 1988, 66(4), 441
53. Prior C.P., Doyle K.R., Duffy S.A., Hope J.A., Moellering B.J., Prior G.M., Scott R.W., Tolbert W.R. , J *Parenter Sci Technol.* 1998, 43(1):15-23.
54. Papadaki M., Bursac N., Langer R., Merok J., Vunjak-Novakovic G., Freed L.E., *Am J Physiol Heart Circ Physiol.* 2001, 280(1), H168
55. Philip E. S., McNeill G.P., Wang J. , *JAOCS.* 1994, 71(12), 1421
56. Rodrigues C.A., Fernandes T.G., Diogo M.M., da Silva C.L,. Cabral J.M., *Biotechnol Adv.* 2011, 29(6), 815
57. Roosta A., Jahanmiri A., Mowla D., Niazi A., Sotoodeh H., *Bioprocess Biosyst Eng*. 2012,Jan 18. [Epub ahead of print]
58. Ramkrishna D., Fredrickson A. G. , Tsuchiya H. M., Biotech Bioeng. 1967, 9(2), 129
59. Riedlberger P., Weuster-Botz D., *Bioresour Technol*.2012, 106, 138
60. Sokól W., *Biotech Bioeng*. 1988, 31(3), 192
61. Silva C.R., Zangirolami T.C., Rodrigues J.P., Matugi K., Giordano R.C., Giordano R.L., *Enzyme Microb Technol.* 2012, 5, 50(1), 35
62. Stoger E., *Biotechnol J.* 2012, Jan 18. doi: 10.1002/biot.201100472. [Epub ahead of print]
63. Stojanović R., Ilić V., Manojlović V., Bugarski D., Dević M., Bugarski B., *Appl Biochem Biotechnol*. 2012 Jan 18, [Epub ahead of print]
64. San Martin R., Bushell D., Leak D.J., Hartley B.S. *Biotec Bioeng*. 1994, 44,21
65. Somrutai W., Takagi M., Yoshida T., *J Ferment Bioeng*. 1996, 81(6), 543
66. Sharon C., Nakazato M., Ogawa H.I., Kato Y., *Ind J. Exp Biol.* 1999, 37, 481
67. Sato K, Kitamori T., Adv Biochem Eng Biotechnol. 2010, 119, 251
68. Suwannakham S., Yang S.T. , Biotechnol Bioeng. 2005, 91(3). 325
69. Tan K. H., Gill C.O.,. Appl. Microb Biotech. 1985, 23(1), 27
70. Tanigaki M., Sakata M., Takaya H., Mimura K., J Ferment Bioeng.1995, 80(4), 340
71. Tosi S., Rossi M., Tamburini E., Vaccari G., Amaretti A., Matteuzzi D. , Biotechnol Prog. 2003, 19(6):1816
72. Timmins N.E., Kiel M., Günther M., Heazlewood C., Doran M.R., Brooke G., Atkinson K., *Biotechnol Bioeng*., 2012 Jan 4. doi: 10.1002/bit.24425.
73. Upadhyaya G., Clancy T.M., Snyder K.V., Brown J., Hayes K.F., Raskin L., .*Water Res.* 2012, 46(4),1309
74. Urban J., Svec F., Fréchet J.M., Biotechnol Bioeng.2012, 109(2), 371
75. Ueno R., Wada S., Urano N., *Can J Microbiol.* 2008, 54(1), 66
76. van der Padt A., Keurentjes J.T.F., Sewalt J.J.W., van Dam E.M., van Dorp, L.J. et al.,

77. JAOCS. 1992, 69(8):748-754
78. Vidmar S., Vito Turk, Igor Kregar , *Appl Microb Biotech*. 1984, 20(5), 326
79. Wei Y., Xu J., Feng Q., Lin M., Dong H., Zhang W.J., Wang C., *J Nanosci Nanotechnol.* 2001, 1(1), 83
80. Wood L.L., Cobbs C.S., Lantz L. 2nd, Peng L., Calton G.J., *J Biotechnol*. 1990, 13(4):305-14.
81. Xudong G.E., Tolosa L., Simpson J. , G.Rao., *Biotech Bioeng*.2003, 84(6), 723
82. Xu Z., Li S., Fu F., Li G., Feng X., Xu H., Ouyang P., Appl Biochem Biotechnol. 2011, Dec 28.
83. Yamamoto Y., Nagahori H., Yao S., Zhang S. and Suzuki E., *J Chem Eng Jpn*., 1996, 29(6),1047
84. Youn J.K., Shang L., Kim M.I., Jeong C.M., Chang H.N., Hahm M.S., Rhee S.K., Kang H.A., *J Microbiol Biotechnol*. 2010, 20(11),1534.
85. Yun C.Y., Liu S., Lim S.F., Wang T., Chung B.Y., Jia Teo J., Chuan K.H., Soon A.S., Goh K.S., Song Z., . *Metab Eng.* 2007, 9(5-6), 406
86. Yang J.D., Lu C., Stasny B., Henley J., Guinto W., Gonzalez C., Gleason J., Fung M., Collopy B., Benjamino M., Gangi J., Hanson M., Ille E. , *Biotechnol Bioeng.* 2007, 98(1), 141