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Application of Fed-Batch Fermentation Modes for Industrial Bioprocess Development of Microbial Behaviour

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Abstract

A major importance features that could be considered during fed-batch fermentation strategy are gaseous and substrate transfer processes, inoculums generation, viscosity, pH, temperature, and all other pathways that made any biochemical, genetic, and morphological changes. Since there are, some products associated with microbial growth and other non-associated products. So a linear relationship between the tested parameter and the final impact should be evaluated through fed-batch fermentation processes. Ideally, the successful scaling-up fermentation system would mean a higher yield product by using low-cost culturing, and low-energy requirements. Since the scaling-up of microbial cells via batch fermentation system is complicated and unpredictable process due to the fluid dynamic generated stresses. The fed-batch system provides a very dynamic environment with large spatial and temporal heterogeneities that can induce multiple physiological responses in microbial cells, depending on the mathematical model of the feeding regime. A maximum specific growth rate is a critical factor indicating the development of any fed-batch fermentation program. Physiological characters of microbial cells are the mandatory step of any development. Since the specific growth rate is a critical parameter restricting maximum possible feed addition in order to avoid substrate accumulations. Such systematic development of fed-batch processes would allow an early assessment of product kinetics, as well as avoiding unnecessary additional cultivations and variations in the final results. By using fed-batch fermentation modes, most of the industrially important bioactive metabolites are produced at optimal or sub-optimal operations conditions. There are numerous examples that can be explained the applications of an optimal fed-batch process were listed in this review.

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Introduction

The key to successful industrial bioprocess development is a statistical experimental design that applied to increase microbial biomass yield and its bioactive metabolites production by minimizing the microbial stress and stimulating metabolic precision [1,2]. Since there are several parameters that influencing the final product quality and quantity [3,4]. These parameters should be studied to finalize the suitable microbial production line [5,6]. Chemical and physical parameters can be characterized preferably depending on fermentation modeling and chemometric analysis at near real-time data collected by online and off-line measurements [2,7]. The best bioprocess development strategy is being identified and optimized initially throughput screening in small scale (shake flasks) to reduce the significant experiments performed by using large batch and fed-batch bioreactor cultures [5,4,8]. So to develop a microbial production process for a strain that has unknown behavior it is essential to determine its physiological characterizations firstly which explained kinetically the relationship between microbial growth and final product [9,10]. Generally, a high initial concentration of microbial cells and low specific growth rate are favorable for maximized the product formation [1,11]. The scaling-up of fed-batch fermentation processes considered as a major problem in any industrial production line [5,12]. Since there are several physical and physiological factors such as a feeding rate, feeding regime, mixing rate, an impeller shape, baffle number, headspace, temperature, pH, cell viability, substrate concentration, and a partial oxygen/carbon dioxide pressures in culture broth should be detected and analyzed briefly [6,13]. In this way, the suitable bioprocess strategy was optimized carefully by controlling these factors. This review article attempts to give an overview of the fed-batch fermentation modes and explains its applications to develop microbial behavior.

Fermentation techniques

Fermentation is a biological system that depended on the microbial conversion of complex structures into simple compounds (metabolites) by using various microbial cells such as Bacteria,

Fungi, Algae, and Actinomycetes [3,7,14]. Fermentation has applied widely to produce different microbial products that have highly beneficent in different fields [5,9,15]. Recently, different fermentation techniques have been modified to maximize microbial productivity that has economic and environmental advantages [16,17]. There are two broad fermentation techniques called submerged fermentation system and solid fermentation system that applied to discover several metabolites (bioactive compounds or structures) by amplifying their production from a laboratory to large scale [6-12]. These metabolites may be antibiotics, enzymes, and phytohormones that have been used in different industries such as pharmaceuticals, agriculture, and food. There are certain bioactive metabolites could be produced highly by using solid-state fermentation whereas other compounds have been scaled-up by using submerged fermentation system [18,19].

Solid-state fermentation system: By using this system, microbial cells utilized solid nutrient-rich waste materials such as grains, Bran, Bagasse, and paper pulp. This simple fermentation system can be used for long fermentation periods since the used substrates were utilized very slowly, and steadily [6,20,21]. The selection of the used substrate(S) was chosen according to their cost and availability [22,23]. There are many industrial bioactive metabolites were produced via solid-state fermentation such as cellulases, pectinases, and proteases [7,9,24]. Since the resulted bioactive metabolites highly vary for each substrate, so it is very important to select the useful substrate for each microbial cell carefully [25,26].

Submerged fermentation system: In this system, the dissolved substrates were utilized rapidly by using growing microbial cells and the produced bioactive metabolites were secreted in the fermentation broth [12,14,16]. This fermentation system is best suited for bacterial cells that required the highest moisture content and produced secondary bioactive metabolites in liquid form [20,22]. The common substrates used in submerged fermentation system are soluble sugars, vegetable juices, and sewage water [25,26].

Fermentation modes

There are three main fermentation modes called batch, fed-batch, and continuous cultivation systems [2,6,12,25]. In general, these modes were regulated by using mathematical models to predict microbial biomass production and final product yield [26,27].

Batch fermentation mode: This mode considered as the simplest lab operation system by using the closed vessel, where all of the medium requirements were added at the beginning of fermentation process to scale-up the microbial biomass production hence its metabolites production [6,18,20]. The microbial metabolites may be produced at a primary or secondary stage of the microbial cultivation period [19,23,28].

Fed-batch fermentation mode: This mode will be started with batch fermentation phase until consumption of one or more substrates, nutrients and/or inducers into a bioreactor; the fresh medium was added by using different feeding regimes [3,10,29,30]. Feeding regime can be added via a fixed volume or variable volume of a fresh medium or substrate only during the time course of the process [10,12,31]. This feeding strategy can be added continuous or exponentially or pulses over a short or long period during the run [5,8,32]. This feeding regime was controlled by feedback control that requires accurate monitoring and operator control to prevent the repressive effects of high substrate concentrations and avoids catabolism repression [10,15,25].

Continuous fermentation mode: This technique has not been widely used in lab scale but common in the industrial scale [33,34]. The feeding regime prolonging the exponential phase by adding the microbial cells with fresh nutrients and the cells are removed from the bioreactor at the specific rate and time [35,36]. There are several control techniques will be used through this mode such as the chemostat restricted by the availability of a limiting substrate, the turbidostat operated under no limitations and the last one include the auxostat since the feed rate controlled a state variable (pH, dissolved oxygen) [33,34]. Therefore, it is necessary to calculate the dilution rate, specific growth rate, a yield of the product by using a number of equations [33,36].

Scaling-up of industrial microbial fermentations

The scale-up strategy is a complex biochemical process for novel microbial production purposes based on the concepts of low production cost, low environmental pollution, and high innovation [3,6,12,37]. This process is scaled-up from shack flask to pilot level to develop the final product and produce sufficient quantities of these compounds [38,39]. Since the small-scale process conditions may not suit for a large-scale production system [7,40]. Therefore, there are various challenges faced during the scaling-up production of microbial metabolites. Since the produced metabolites have been discovered for application in pharmaceutical, agriculture and food industries [12,41]. Bioprocess development starts from milliliter scale cultivations to determine the optimal cultivation factors (physical and chemical parameters) that may be used for the following large-scale production to get the whole process development procedure to rely on the specific microbial response [2,4,31,42]. In this stage, it can be determining the biological responses and essential nutritional requirements at all incubation periods that may be used to reduce a few stages for a scaling-up program as possible [43,44]. An understanding of the biochemistry of the microbial metabolites pathway is extremely important for scaling-up programs [10,12,45]. There are many parameters should be considered when change scale production or fermentation modes such as availability of nutrient source, metabolites compartmentalization, agitation, aeration, fermenter broth capacity and sterilization method, that may be useful to improve a scaling-up strategy and achieve a final product quality [46,47]. Shake flask scale stage (mini-fermenter) is a useful screening stage for starting the evaluation of raw materials variability (inorganic, organic or mixed sources), cultivation systems, temperature ranges, pH, inoculums size, and the microbial responses to high/low agitation conditions. Since these data may be support input into further evaluation in bioreactor tanks [12,20,30,48]. Bioreactors are a sophisticated version of shake flask system with the option of a computer controlling of gas supplying, pH regimes, and feeding nutrients [20,21,49,50]. Scaling-up processing for recovery the natural product may be evaluated by using bench-top or pilot scale models as summarized in Figure 1.

Bioreactors hardware configuration and culture techniques

The conversion of nutrients into desired metabolites via microbial cells is achieved in the bioreactor that considered as a heart of all bioprocess operations. It provides a well-monitored system to achieve an optimal growth environment and natural product formation [51,52]. There are several types of bioreactors are designed for each microbial fermentation processes that varied from internal mechanical agitation to air bubble columns and loop bioreactors [2,53,54]. Recently, there are new types of designed bioreactors such

Figure 1: Scale-up bioprocess development set-up for microbial product from 500 ml; shake flask, 1 L, 9 L, 15 L, and 70 L to 300 L stirred bioreactors.

as Photobioreactors, Fluidized bed bioreactors, and Packed-bed bioreactors [55,56].

Continuous stirred tank bioreactor: The most common bioreactor type is the stirred tank bioreactor that used for scaling-up fermentations of bacterial, fungal and actinomycetes cells [12,20,43,57]. A bioreactor equipped with an impeller, baffles, and other mixing devices that formed an idealized agitation to provide efficient mechanically mixing under controlled pH, Temperature, airflow, and dissolved oxygen [58,59]. A bench top stirred bioreactor tank that shown in Figure 2 was used for scaling up production of different microbial cells at City of Scientific Research and Technological Applications, New Borg El-Arab City, Alexandria, Egypt. Typically, it consists of the cylindrical glass vessel that fitted with a mechanically rotating shaft that has two impellers and four baffles. There are several stirred impellers patterns were selected depended on the tested microbial cell that produced radial-flow of axial-flow.

Bubble columns and airlift loop bioreactors: Nevertheless, the bubble columns and loop bioreactors were used for cultivations of algal cells and mammalian cells respectively that sensitive to mechanical agitation [50,54,60-65]. These bioreactors considered as an attractive alternative to stirred tank bioreactors that used especially for scaling up production of bioactive metabolites that extracted from some microbes such as yeast and algae [11,66-68]. Since these bioreactors are constructed as cylindrical vessels that supplied with gas sparger at their bottoms to form gas bubbles. Sparging gas bubbles in the liquid medium at lower input levels leads to convective flows within the entire vessel [50,54]. Therefore, these simple and cost-effective bioreactors can be built at larger volumes than stirred bioreactors, which used for large-scale industrial applications [15,35,54,62].

A photobioreactor: This bioreactor has a light system that used for cultivation of phototrophic microbial cells such as macro-algae, microalgae, cyanobacteria, and purple bacteria [11,67,68]. This bioreactor should have specific artificial environmental conditions that controlled carefully to generate the microbial biomass by using light and carbon dioxide.

Fluidized bed bioreactors: This type of bioreactor device can be used to complete a variety of multiphase of chemical reactions by using biocatalysts such as enzymes or microbial cells [69,70]. Since in this bioreactor a liquid material or gas is passed via a solid granular material to suspend the solid that was a fluid [71,72]. This bioreactor considered as complicated bioreactor but now used in many industrial applications [73,74].

Packed bed bioreactors: This bioreactor considered as a promising tool for tissue engineering applications that supporting various cell lines for long incubation periods due to the immobilization of cells within matrices [51]. This technique is being employed in studies to develop the bioartificial liver and bone marrow cell systems [75,76].

Up-stream and down-stream processing

Up-stream processes are the first steps that performed for grew the biomolecules of the tested microbial cells in bioreactor via batch and fed-batch fermentation modes. In this process, all operating parameters that include medium composition, fermentation mode, feeding rate, and scaling-up strategy were developed [76,77]. In addition, the optimal ranges for pH, Temperature, Foam type, Dissolved oxygen rate, agitation rate, and sterilization management were studied [78,79]. The last sections of this bioprocess called downstream processes by which the final products were harvested and analyzed. The upstream and down-stream processes were summarized in Figures 3 and 4. Throughout a cultivation run, the most operating parameters should be kept nearly at a constant set point by monitoring and control the process variables such as temperature, pH, dissolved oxygen and foam level [80,81]. The temperature maintains at the set point by regulating the cooling water in the bioreactor via water chillier [82,83]. By using peristaltic pumps that connected to the bioreactor, the pH was regulated at the set point by pumping the base and acid solution [70,84]. The dissolved oxygen is regulated through microbial cultivation by increasing the airflow rate or by increasing the agitation speed [12,67,83]. Since the oxygen levels were controlled by cascading the impeller speed and gas rate that calculated in Volumes air/Volume of broth (vvm) [83,84]. A foam was formed by increasing the gas-liquid interface via the broth medium and the excessive foaming should be avoided because it reduced the microbial productivity by adding an anti-foam agent [75,82].

The physiological response of microbial cells to the fed-batch fermentation mode

This cultivation mode has become an extremely important technique used through industrial biotechnology field in order to increase the microbial cells density [84,85]. Therefore, it is important to understand the scaling-up process governed by important considerations (culturing system, culturing conditions, and vessel parameters) [12,86]. Since there are, some microbial products did not perform at the large-scale process as well as the small-scale process [72,87,88]. Previously, it is observed at a large-scale production of microbial cell and/or their associated products such as *E. coli*, *Bacillus subtilis*, and *Saccharomyces cerevisiae*, since the biomass yields were found to be lower than that produced via a small-scale process [10,18,55,60,89,90]. Therefore, the successful fed-batch fermentation mode was depended on the optimization of the main engineering considerations to control the microbial behavior by using an optimal bioprocessing strategy [91,92]. The salient feature to increase volumetric productivity is the feeding system of the substrates or completely required nutrient. The feeding regime was controlled by impeller maxing, catabolic regulation, heat generation, and oxygen consumption during the fed-batch fermentation system to avoid the overflow metabolic routes by using a slowly metabolized carbon source such as glycerol [37,55,56,59]. Previously, the overflow metabolism has been reported for some microbial cells such as *E. coli*, *Bacillus subtilis*, and *Saccharomyces cerevisiae* at glucose concentration above

Figure 2: A Biofo 310 fermentor; 7 L (New Brunswick Scientifc, Edison, NJ, USA).

30 mg/ml [10,20,21]. Finally, to control the fed-batch fermentation process the online and off-line measurements should not be shown changes in those being measured kinetically [26,30,37]. Stress responses are complex networks of regulatory systems that induced to help microbial cells to adapted or survive under sub-optimal growth conditions [15,70,81]. Until now, any physiological responses of the tested microbial cells within bioreactors were measured indirectly by calculating the external factors surrounded the microbial cells such as carbon source concentration, oxygen limitation, and overall biomass yield [84,85]. Since these changes often resulted in the transcription and expression of stress proteins which include the inhibition of DNA replication initiation, a reduction in rRNA synthesis, glycolytic activity, DNA metabolism, protein production, and the synthesis of structural components that reduced allowing cells to survive at sub-optimal conditions leading without doubt too low biomass productive at large-scale [81,82]. The microbial growth yield is one of the main stoichiometric variables via catabolic and conserved carbon source as defined in equation (Equation 1). [80-83].

$$Y = -\frac{\delta X}{\delta S} \approx -\frac{\Delta X}{\Delta S} \quad (1)$$

Where; ΔX is the increase in microbial biomass weight that indicated the utilization of the used substrate (ΔS) that can vary in time. The negative sign is introduced because δX and δS vary in opposite senses.

There are two groups of substrates will be used for microbial growth called catabolic and conserved substrates which are sources of biogenic material that used for producing cellular material [15,73,89]. The catabolic substrates (energy materials) such as hydrogen ions for lithotrophic hydrogen bacteria, oxidizable or fermentable organic materials were used for heterotrophic bacteria and fungi, and soon [55-59]. The conserved substrates (non-carbon sources of biogenic elements) are incorporated into *de novo* synthesized cell components that being conserved to biomass [30-37]. The economic coefficient expresses explicitly the nutrient requirements for microbial growth, i.e., how many mass units of a particular substrate should be consumed to produce one unit mass of cell material. Therefore, the growth efficiency depends on the partitioning of consumed materials to produce new cell and extracellular bioactive metabolites [50-54]. Since the mass balance can be calculated by using the following equation (Equation 2).

$$\delta E_S = \delta E_X + \delta E_P \quad (2)$$

In addition, some catabolic substrates could be used as a source of biogenic materials that can assess to measure the final yield [80,81]. Since Y_E is a biomass yield per mass unit of oxidized substrate, and Y_A is a biomass yield per mass unit of an assimilated substrate [82,83]. A total carbon consumed equals C incorporated into cell plus C oxidized to CO_2 to provide energy plus C incorporated into by-products as shown in Equation (Equation 3) [79,80,85]. When the extracellular microbial metabolites can be re-used, the C_S is equivalent to the δC_P, so its value was neglected then the δC_X balanced the substrate (Equation 4).

$$\delta C_S = \delta C_X + \delta C_{Co2} + \delta C_P \quad (3)$$

$$\frac{1}{Y} = 1 + \frac{1}{Ye} \quad (4)$$

Modeling of the microbial activities via kinetic growth parameters

Software sensors were used to control the physiological parameters of microbial cells via sophisticated process based on the real-time value of specific growth rate, substrate consumption, temperature, pH, oxygen supply, and biomass production [83,84]. Microbial growth can be identified as an increase of the microbial mass weight or cell number per unit time in the presence of a suitable medium and the best culturing conditions [26,70]. The microbial growth kinetics can be described by using the microbial growth pattern that cultured in a bioreactor [55,57]. A typical microbial growth curve includes four phases that called Lag phase, Logarithmic phase, Static phase, and Death phase [85-87]. In the lag phase, the microbial cells need to be adapted to the new culturing conditions so there is no growth was observed [20-25]. Then, the highest cellular growth rate was detected by using the concentration of substrate that consumed rapidly in Logarithmic phase. After that, the microbial cell number will get constant in the static stage. Death phase is the last growth stage since the microbial cells decreased due to the depletion of essential nutrients and the accumulation of toxic materials [23,26,82]. The biochemical activities of the tested microbial cells may be determined kinetically by using biomass production (dry mass weight or cell count), oxygen consumption, carbon dioxide formation, and ATP production [21,35,80]. The microbial growth rate can be detected by using the microbial generation time (Equation 5).

$$G = t/n \quad (5)$$

where G is a generation time, n is a number of generations, and t is time.

Kinetics Greek word means forcing to move, in microbiological studies deals with the mode of action and rate of physical and chemical processes of the microbial cells. Since microbial kinetics covered all microbial lifestyles such as the microbial growth cycles, production formation, environmental conditions, adaptations, biological interaction, and mutations [81,82]. The growth of the microbial cells can be explained and optimized by using different mathematical kinetic models such as Blackman, Monod, and Moser [73,80,84]. Since there are, various statistical programs have been used for detected and optimized of these parameters such as Matlab, Sigma plot, and Statistica programs. The Blackman model used the earliest simple nonmathematical method that describes the microbial growth by using (Equation 6). This model makes a sharp changeover from first order to Zero-order (not for gradual transition) only at high substrate concentration [70,82,90]. Monod model predicted the

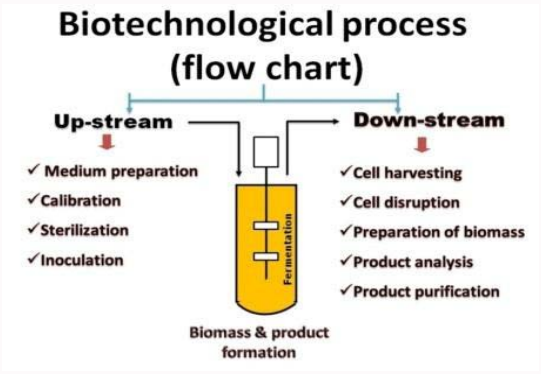

Figure 3: Flow chart of upstream and downstream parameters that may e used for bioprocessing development of microbial cells.

non-linear relation between specific growth rate and low substrate concentration [50,42,82]. The Monod equation formulated in (Equation 7).

$$\mu = \begin{cases} \dfrac{\mu_m . S}{K_S} & if \quad S < K_S \\ \mu_m & if \quad S \geq K_S \end{cases} \quad (6)$$

$$\mu = \frac{\mu_m . S}{K_S + S} \quad (7)$$

where S is the concentration of nutrient at time t and μ_m is the maximum specific growth rate.

This model describes strongly the μ_m for low S and slowly for the highest S until saturation state, since K_s is a half-saturation constant as shown in (Equation 8). Finally, the maximum specific growth rate must be less than 1 and cannot be negative [30,43,82]. Hermann Moster model was a modified way of Monod model to describe the microbial mutation so this model was never popular [50,67,82]. Its mathematical formulation of the Moser model is given by (Equation 9).

$$\mu = \frac{\mu_m}{2} \; ; \; S = K_S \quad (8)$$

$$\mu = \frac{\mu_m S^n}{K_s + S^n} \quad (9)$$

where n is the adjustable factor. This model can predict interesting dynamic behaviour flexibility in continuously stirred bioreactors. The combination of different mathematical modeling provides a meaningful interpretation of the results by using new aspects for microbial physiology. The best practice in bioprocess development was depended on different biomass growth and its metabolites production kinetics [80-84]. Since controlling, the specific growth rate (μ) in a decreasing value over the cultivation process is preferred to maximize the final product. The optimum specific growth rate is a critical variable that enhancing product formation as well as affecting the product quality [20-24]. The production kinetics is a relationship between specific productivity and the specific growth rate per-process time that can be calculated similarly for batch and fed-batch fermentation systems as shown in (Equation 10) [73,80,83].

$$\mu(t) = \frac{In(X * V) - In(X_0 * V_0)}{(t - t_0)} \quad (10)$$

Where, X is a cell concentration, X_0 is an initial cell concentration, V is the final broth volume and V_0 is the initial broth volume at measuring time t and initial time t_0 respectively. Yield coefficient was calculated by using (Equation 11).

$$Y_{X/S} = \frac{\Delta X}{\Delta S} = \frac{X - X_0}{S_0 - S} \quad (11)$$

where: $Y_{X/S}$ Biomass yield on a substrate, X Cell concentration, X_0 Initial cell concentrations, S Substrate, S_0 Initial substrate concentration. X and X_0 are biomass concentrations (g/l) at measuring time and initial time t_0 respectively. S and S_0 are the consumed amounts of carbon source (g/l) at the same times mentioned previously [72,75,80]. In the logarithmic growth phase, cell biomass density increases exponentially with time and specific growth rate μ (h^{-1}) is independent of nutrient concentration as shown in (Equation 12).

$$\frac{d_x}{d_t} = \mu X, \; X = X_0 \quad at \quad t = 0 \quad \rightarrow \quad ln\left(\frac{X}{X_0}\right) = \mu t, \quad or \quad X = X_0 e^{\mu t} \quad (12)$$

The most common kinetic model for cell growth was used to determine the maximum specific cell growth rate (Equation 13). Finally, if the specific growth rate μ is constant; the feeding rate calculated by using (Equation 14).

$$\mu = \frac{\mu_{max}}{K_s} + S \quad (13)$$

where; μ specific cell growth rate (hr^{-1}), μ_{max} maximum specific cell growth rate (hr^{-1}), S substrate concentration (g/l), K_S Saturation constant (g/l) = S when $\mu = 1/2\ \mu_{max}$.

$$XV = X_0 V_0 e^{\mu t}, \quad \frac{d(SV)}{dt} = 0,$$
$$\frac{d(SV)}{dt} = FS_0 - \left(\frac{\mu XV}{Y_{X/S}}\right),$$
$$F = \frac{\mu X_0 V_0 e^{\mu t}}{S0 Y_{X/S}} \quad (14)$$

where, F is the feed flow rate (Lh^{-1}), S_o is the concentration of limiting substrate in the feed (g/L), μ is the specific growth rate (h), X is the biomass concentration (g/L) and Y is the yield of cells on the substrate (g biomass/g substrate).

The cost-effective statistical experimental design used for optimization strategy

There is a strong relation between the medium compositions and maximum biomass production [1-5]. Since the accumulations of microbial cells and its bioactive metabolites are influenced strongly by carbon/nitrogen sources, inorganic salts, and trace elements [10,11,22,23]. Therefore, to achieve the highest microbial mass weight, it is necessary to select the proficient microbial medium and cultivation conditions [29,31,32]. For industrial fermentation mode, the picking of an excellent microbial medium depends on its availability on the local market and its price [1,5,6,10-14]. To detect the optimum medium components and the microbial culturing conditions, the optimization strategy was used carefully [31,32,73]. Since there are non-statistical (one-factor-at-time) and statistical optimization (experimental designs) methodologies can be applied [53-61,84]. Recently, statistical experimental designs considered as powerful tools in order to it can overcome obstacles, since by which the significant variables can be checked and studied their complex interactions [10,11,53,54]. The most common statistical experimental designs called Plackett-Burman, Box-Behnken, and Robust Taguchi

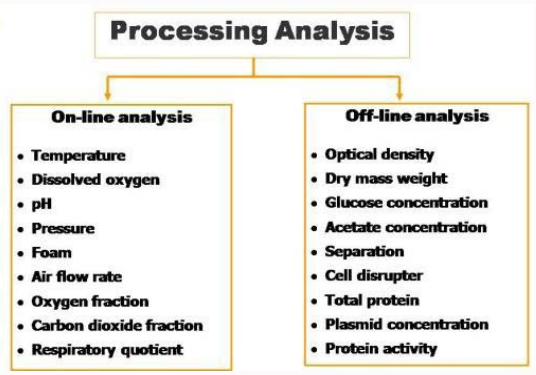

Figure 4: Flow chart of on-line and offline analysis that may e used for bioprocessing development of microbial cells.

designs that applied by using Minitab or Sigma Plot programs [33,55,59]. This fractional factorial method (Plackett-Burman design) was selected for screening all tested variables to select the most significant factors that affect the microbial cells or culturing condition (Equation 15) [31,60,73].

$$Y=\beta_0+\Sigma\,\beta_i X_i \qquad (15)$$

where Y is a response, β_0 is a model intercept, β_i is the estimated factor, and X_i represents the variable [53-61]. This design followed by the response surface method (Box-Behnken design) that applied to detect the optimum quantities for each factor and study the interactions among them (Equation 16).

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{11}X_1^2+\beta_{22}X_2^2+\beta_{33}X_3^2+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3 \qquad (16)$$

where β_1, β_2, and β_3 are linear coefficients; β_{12}, β_{13} and β_{23} are cross product coefficients; and β_{11}, β_{22} and β_{33} are the quadratic coefficients.

Robust Taguchi design was set up by using several stages to gain the desired microbial mass weight by calculating signal-to-noise (S/N) ratio for each factor [84]. The S/N ration can be determined by using three categories that called nominal is the best characteristic, that may be smaller, or larger [23,73].

Development of fed-batch fermentation strategy for efficient microbial behavior

Fed-batch, fermentation systems are widely applied because they have the advantages of batch and continuous fermentation systems [10,18,20]. Fed-batch fermentation mode is started as batch fermentation mode until the substrate was consumed then the feeding initiated continuously. The main advantages of the fed-batch fermentation operation system are the control of microbial growth rate, bioactive metabolites, and limitation of oxygen transfer by inlet feeding rate [23,30,37]. The fed-batch fermentation system can be operated by manual and automatic monitoring process since this system is usually run with a calculated feed profile according to the types of microbial cells and their nutritious (sugar, nitrogen, and phosphate sources) [29,31,32]. Various nutritional, environmental factors and physicochemical properties should be developed carefully for industrial applications [73,84]. Therefore, this review summarizes enormous available reports of the development of fed-batch fermentation modes to produce the highest microbial biomass weight that formed bioactive metabolites. Since the eco-friendly bioactive metabolites such as enzymes, antibiotics, vitamins, alkaloids, phenols or any other biochemical compounds can be extracted from bacterial, Actinomycetes, fungal, and algal cells [55-59]. This section attempts to list some of the bioactive compounds extracted from different microbial cells to get the better fermentation technique for a successful industrial production line.

Algal bioactive metabolites: During real fermentation run, the variables manipulations were monitored to compare the inputs values and the predicted outputs data (structured model) that calculated by using the on-line and off-line measurements [11,67,68]. Since, the structured model has described kinetically the effects of dissolved oxygen, carbon dioxide production or consumed viscosity, temperature, pH, feeding rate, feeding regime, and consumption of carbon/nitrogen sources. Since there are, many reports used some micro-algae and macro-algae to produce lipids, oils, and carbohydrates that suitable for industrial production of biodiesel, biogas, or bio-ethanol via fed-batch fermentation system [11,67,68]. For the economical production of biofuel, Devasya (2017) used batch and fed-batch fermentation cultivation systems for microalgae (blue-green algae) such as *Nannochloropsis gaditana* to produce some lipids that suited for biodiesel production. Since in batch phase the produced biomass was recorded as 2 g/l by using road salt media [70]. Then the controlled feeding of nitrates via fed-batch cultivation was applied by using pulse, continuous, and staged feeding regimes. A maximum lipid fraction was achieved at pulse-fed cultures 58.3%. The other two feeding modes (continuous and staged) were not as effective for lipid and biomass production by using *Nannochloropsis gaditana* cells at pH 11 and above. De Swaaf et al. [71]. Produced the Docosahexaenoic Acid (DHA), a polyunsaturated fatty acid with food and pharmaceutical applications, during batch cultivation on the complex by using the heterotrophic marine alga *Crypthecodinium cohnii via* fed-batch cultivation. Since glucose and acetic acid were examined as carbon sources. For both substrates, the feed rate was adapted to the maximum specific consumption rate of *C. cohnii*. In glucose-grown cultures, this was done by maintaining a significant glucose concentration (between 5 and 20 g/L) throughout fermentation. In acetic acid-grown cultures, the medium feed was automatically controlled via the culture pH. A feed consisting of acetic acid (50% w/w) resulted in a higher overall volumetric productivity of DHA (rDHA) than a feed consisting of 50% (w/v) glucose (38 and 14 mg/L/h, respectively). Tang et al. [72] solved the shear stress (pH, and dissolved oxygen tension) that affected on scaling-up the fed-batch fermentation of the medicinal mushroom *Ganoderma lucidum* from a shake flask to a stirred tank bioreactor. The maximal biomass (22.62 g/L), intercellular polysaccharide production (4.74 g/L), and ganoderic acid production (798.0 mg/L) were attained at a low impeller tip speed of 1.234 m/s. Finally, the fed-batch fermentation of *G. lucidum* was successfully step-wise scaled-up from 7.5 to 200-L stirred-tank bioreactor [68-72,80,87]. In addition, there is heterotrophic marine algal cells were used to produce docosahexaenoic acid and a polyunsaturated fatty acid with pharmaceutical applications by using sea salt, yeast extract as a nitrogen source and glucose as a carbon source [24,67,77,79]. *Via* fed-fermentation mode, the culture viscosity and vigorous maxing were controlled to sustain aerobic conditions by feeding rate in order to maximize the algal cells than their bioactive metabolites productivity [82,89].

Bioactive metabolites extracted from streptomyces cells: Dzhavakhiya et al. [74] Produced Virginiamycin by a mutant

Streptomyces virginiae as VKM Ac-2738D strain from shake flasks to a pilot-scale (100 L) stirred fermentor. Since a natural mix of macrocyclic peptide lactones M and S is widely used in the industrial production of ethanol fuel and as an antibiotic feed additive for cattle and poultry. After the optimization of pH and dissolved oxygen concentration (6.8-7.0 and 50%, respectively), the fed-batch fermentation of VKM Ac-2738D with continuous addition of 50% sucrose solution (5 g/L/day starting from 48 h of fermentation) resulted in a final virginiamycin titer of 4.9 g/L. The developed technology has several important advantages, which include (1) the optimum M1:S1 ratio in the final product, (2) the possibility to use sucrose as a carbon source instead of traditionally used and more expensive glucose or D-maltose, and (3) selective binding of up to 98.5% of produced virginiamycin on the adsorbing resin. To reduce the upstream and downstream costs and to increase the final volumetric productivities of different antibiotics produced by using *Streptomyces* sp, the fed-batch culturing modes were applied at bioreactor levels [22,29,92]. Since the effect of feeding rate and glucose concentrations were determined kinetically. However, in some cases, the higher glucose concentrations supported the highest biomass production but reduced antibiotic production [22,29,32]. So the antibiotic productivity was considered as a major responsibility for the successful fed-batch cultivation system [56,62]. In this case, the fed-batch experiment may be designed by using mono-glucose feeding and/or complete medium feeding to maximize the antibiotic production by cascading the dissolved oxygen value at 40% to 60% with feeding pump [22,29]. Recently, there are many nanobiotechnological strategies, can be used as proficient tools to improve the bioactive metabolites production by reducing the production cost [10,22,29,84]. Since bad construction of the optimization techniques affecting the results, so statistical experimental designs were applied as proficient and more effective methodologies to evaluate the desired products [10,11].

Bacterial bioactive metabolites: The recent trend for producing new natural bioactive metabolites has discovered by using industrial biotechnological strategies [18,20,26]. Since there are variations among different fermentation systems and modes, so a lot of exploration still needs to be applied to maintain the microbial natural product and quality and to reduce the final products cost [48,55]. The simplest microbial cells that used for scaled-up natural products (enzymes, lipids, and carbohydrates) industrially are the bacterial cells via submerged fermentation systems [56,61,62]. However, some workers reported recently that solid fermentation system is more efficient than submerged fermentation system because this system can be attributed to the metabolic differences since the accumulation of intermediate metabolites lowered some enzyme activities and production efficiency [18,20,62,71]. El-Sedawy et al., 2012, studied the production of rifamycin B using the gene amplified variant of *Amycolatopsis mediterranei* (NCH) was initially optimized in shake flasks through medium modifications and fed-batch addition of uracil [9]. Since the yield was increased by 21.7% (from 11.7 to 14.3 g/l) when F2 m1 medium was used. The production was further verified and optimized in fed batch-mode in a laboratory fermentor using F2 and 3 medium and the optimized conditions (agitation 500 rpm, aeration; 1.5 for 3 days then control DO at 30% thereafter, pH; 6.5 for 3 days and 7 days thereafter and control a temperature at 28°C). Fed batching of glucose syrup (5% v/v at day 3) and glucose (1% at days 6 and 8) increased the yield from 17.8 to 20.9 g/l (17.3%) at 10 days. A yield of up to 20 g/l was recorded when 0.1% uracil was fed-batched at day 2. Integration of the most optimum conditions for fed-batching glucose syrup, glucose and uracil further increased the yield from 17.8 to 24.8 g/l (39%) in 10 days. The overall optimization of rifamycin B production increased the yield of almost two folds. Statistical analysis revealed that there is a significant increase in rifamycin B production by using one-way ANOVA at $p<0.05$ in all the tested fed-batch addition regimes. The Bacterial Ghost (BG) platform technology evolved from a microbiological expression system incorporating the φX174 lysis gene E. E-lysis generates empty but structurally intact cell envelopes (BGs) from Gram-negative bacteria, which have been suggested as candidate vaccines, immunotherapeutic agents or drug delivery vehicles. The development of both economic and robust fed-batch production process for BGs required a toolset capable of dealing with rapidly changing concentrations of viable biomass during the E-lysis phase *via* Meitz et al. [76], (2016) study. This challenge was addressed using a transfer function that was implemented to a feed-controller, which followed the permittivity signal closely and was capable of maintaining a constant specific substrate uptake rate during lysis phase. So, the yield of BG production processes by a factor of 8-10 when compared to currently used batch procedures reaching lysis efficiencies >98%. This provides elevated potentials for commercial application of the bacterial ghost platform technology. Ibrahim and Chel [77] (2010), were employed different fermentation strategies for the cultivation of a new poly(3-hydroxybutyrate)- accumulating *thermophilic bacterium*, *Chelatococcus* sp., strain MW10, with the aim of achieving High Cell Density (HCD) growth and high poly (3-hydroxybutyrate) [poly(3HB)] productivity. Enhanced cultivation was achieved by a Cyclic Fed-Batch Fermentation (CFBF) technique (42-liter scale). Park et al. [78], (2011), used the bacterial ghost system of *E. coli* K12/pHCE-InaN-GAPDH-ghost 27 SDM that created for mass production of a *Streptococcus iniae* ghost vaccine. The optimal fed-batch process for high cell density culture of *E. coli* K-12/pHCE-InaN-GAPDH-ghost 27 SDM was developed using the nutrient feeding strategy with riesenberg defined the medium. Fermentation was conducted in four phases as follow: (1) initial batch phase, (2) fed-batch phase for high cell density culture, (3) thermal induction phase for the formation of a ghost by the expression of lysis gene E, and (4) high temperature holding phase to increase ghost formation efficiency. The maximum Ghost Bacteria Vaccine (GBV) was obtained from the fed-batch fermentation of 34.9 g Dry Cell Weight (DCW)/L. Therefore, this GBV is proposed as an effective vaccine in aquaculture for the prevention of *streptococcal* disease. Now, there are a multitude of antibiotics (cyclosporins, tetracyclines, surfactins, streptomycin, and cephalosporin) that have produced commercially using fed-batch fermentation systems by using bacterial cells relied on the development of suitable substrates and nitrogen source [20,64,71]. In addition, there are some peptides such as antihypertensive peptides that used to inhibit the activity of the angiotensin enzyme for blood pressure regulation were produced and developed kinetically by using lactic acid bacteria such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii spp. Bulgaricus via* solid and submerged fermentation techniques [2,7,62].

Fungal bioactive metabolites: It is well known that high-viscosity fermentation broth can lead to mixing and oxygen mass transfer limitations [90-95]. The seemingly obvious solution to this problem is to increase agitation intensity. In some processes, this has been shown to damage mycelia, affect morphology, and decrease product expression. However, in other processes increased agitation shows no effect on productivity [92]. While a number of studies discuss morphology and fragmentation at the laboratory

and pilot scale, there are relatively few publications available for production-scale fungal fermentations [96-99]. Li et al., assessed morphology and fragmentation behavior in large-scale, fed-batch, fungal fermentations used for the production of protein [96]. To accomplish this, a recombinant strain of *Aspergillus oryzae* was grown in 80 L fermenters at two different gassed, impeller power-levels (one 50% greater than the other). Impeller power is reported as Energy Dissipation/Circulation Function (EDCF) and was found to have average values of 29.3 ± 1.0 and 22.0 ± 0.3 kW/L s^{-1} at high and low power levels, respectively. Morphological data show hyphal fragmentation occurred by both shaving-off, of external clump hyphae and breakage of free hyphae. Therefore, this behavior was produced due to the slow growth of the culture during this fed-batch process. Bhargava et al. [98], was determined if pulsed addition of substrate could be used to alter filamentous fungal morphology during fermentation, to result in reduced broth viscosity. In all experiments, an industrially relevant strain of *Aspergillus oryzae* was grown in 20 L fermentors. As a control, cultures were fed limiting substrate (glucose) continuously. The same total amount of glucose was fed in repeated 300-s cycles, with the feed pump on for either 30 or 150 s during each cycle. Variables indicative of cellular metabolic activity (biomass concentration, oxygen uptake rate, base consumed for pH control) showed no significant difference between continuous and pulse-fed fermentations. In addition, there was no significant difference between total extracellular protein expression and the apparent distribution of these proteins. In contrast, fungal mycelia during the second half of pulse-fed fermentations were approximately half the size (average projected area) of fungi during fermentations with continuous addition of glucose. As a result, broth viscosity during the second half of pulse-fed fermentations was approximately half that during the second half of continuous fermentations. If these results prove to be applicable for other fungal strains and processes, then this method will represent a simple and inexpensive means to reduce viscosity during filamentous fungal fermentation. Industrial filamentous fungal fermentations are operated typically in fed-batch mode since an oxygen control represents an important operational challenge due to the varying biomass concentration. Bodizs et al. [99], controlled the dissolved oxygen by manipulating the substrate feed rate. Since the regulation of dissolved oxygen using a cascade control scheme that incorporates auxiliary measurements to improve the control performance. The computation of an appropriate set point profile for dissolved oxygen is solved via process optimization. Experimental results obtained at the industrial pilot-scale level confirm the efficiency of the proposed control strategy but also illustrate the shortcomings of the process model at hand for optimizing the dissolved oxygen set points. Also, there are several bioactive metabolites such as natural antioxidants, biopharmaceutical production, sophorolipids, invertase production, pectinesterases, polygalacturonases, phytase, and β-carboline alkaloids that have industrial importance could be produced previously by using different microbial cells via solid and submerged fermentation strategies [46,74,76].

Conclusion

Fermentation has applied widely to produce a wide variety of natural products (bioactive metabolites) that have highly beneficent in different fields. Recently, different fermentation techniques have been modified to maximize microbial productivity that has economic and environmental advantages. Since there are, two broad fermentation techniques called submerged fermentation system and solid fermentation system that applied to discover several bioactive compounds or structures by amplifying their production from a laboratory to large scale. The scaling-up strategy is a complex biochemical process for novel microbial production purposes based on the concepts of low production cost, low environmental pollution, and high innovation. This process is scaled-up from shack flask to pilot level to develop the final product and produce sufficient quantities of these compounds. There are three main fermentation modes called batch, fed-batch, and continuous cultivation systems. In general, these modes were regulated by using mathematical models to predict microbial biomass production and final product yield. Fed-batch mode will be started with batch fermentation phase until consumption of one or more substrates, nutrients and/or inducers into a bioreactor; the fresh medium was added by using different feeding regimes. Feeding regime can be added via a fixed volume or variable volume of a fresh medium or substrate only during the time course of the process. This feeding strategy can be added continuous or exponentially or pulses over a short or long period during the run. This feeding regime was controlled by feedback control that requires accurate monitoring and operator control to prevent the repressive effects of high substrate concentrations and avoids catabolism repression. For a long-term perspective, applications of dynamic fed-batch feeding strategies are very promising and time-saving. Generally, a high initial concentration of biomass and low specific growth rate during production are favorable to grow microbial cells rapidly to get a high biomass concentration. To reduce workload and enhance product quality by controlling the growth/culturing conditions, the software-sensors could be used. A deeper understanding of the underlying concepts of process comparison and development has become increasingly relevant, as new genetic constructs are made available. There are various nutritional, environmental factors, and physicochemical properties that affecting the production line of microbial cell and/or its bioactive metabolites *via* fed-batch fermentation systems should be developed carefully for industrial applications. Since the eco-friendly bioactive metabolites such as enzymes, antibiotics, vitamins, alkaloids, phenols or any other biochemical compounds can be extracted from bacterial, actinomycetes, fungal, and algal cells. The fed-batch mode can be operated by manual and automatic monitoring process since this system is usually run with a calculated feed profile according to the types of microbial cells and their nutritious (sugar, nitrogen, and phosphate sources). For industrial fermentation mode, the picking of an excellent microbial medium depends on its availability on the local market and its price.

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