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Principles and Case Studies of Fed Batch Fermentation and Continuous Fermentation

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Abstract: Modern fermentation processes include a variety of fermentation methods, such as fed batch fermentation and continuous fermentation. This paper will focus on the principles and case studies of these two methods. Fed batch fermentation originates from fractionation fermentation with closed culture and adjustment of the pH value of the carbon source, to which the process of feeding the carbon source to the cell culture in a controlled manner has been added. This type of fermentation is more commonly used compared to the other. Continuous fermentation is also a closed fermentation system, which can operate without restrictions by continuous or intermittent addition of fresh nutrient media to the fermenter; however, it is susceptible to contamination by stray bacteria and metabolic inconvenience.

Keywords: Fed batch fermentation; Continuous fermentation; Fermentation process

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1. Background

Modern fermentation engineering refers to a technology that exploits the characteristics of biological cells and applies fermentation principles on an industrial scale through modern engineering methods. Fermentation has been widely practiced in the world of food brewing as early as when people began having awareness about the concept of microorganisms. Beer, wine, bread, and cheese in the West, lactic acid fermentation products in the Middle East, as well as sauces and soy sauce in the East are all examples of human-made natural fermentation of foodstuffs [1]. The rigid demand for wartime resources such as acetone and penicillin during World War II greatly aided the industrialization of the fermentation process; the culture methods used for penicillin and acetone at that time often faced problems of insufficient yield to meet the military's needs and susceptibility to contamination by contaminants, while researchers from Canada, the United States, and other countries adopted the pressurized steam sterilization technology. Researchers from Canada, the United States, and other nations utilized pressured steam sterilization, aseptic inoculation, and cylindrical steel fermenters with agitation and air to address the problem of contamination by waste contaminants. Simultaneously, the researchers established and enhanced indices of fermentation broth, pH, temperature, and nutrients during fermentation for better solid yield management [1].

The fermentation process necessitates the development of a medium for culturing the processing organism during inoculum preparation and in the production fermenters, as well as the sterilization of the medium, fermenters, and auxiliary equipment, and a sufficient amount of active pure culture to be inoculated into the production vessels [2]. We will explore two fermentation techniques in this article – fed batch fermentation and continuous fermentation – as well as their underlying concepts and results in practice.

2. Distinguishing the characteristics of different fermentation systems

Different fermentation strategies are practiced based on yield maximization. The more common ones are fed batch fermentation and continuous fermentation. In this paper, these two fermentation systems will be included in the discussion.

2.1. Fed batch fermentation

Batch fermentation is a closed culture system, as only a limited amount of sterilized nutrient media is introduced into the fermenter. The medium is inoculated with appropriate microorganisms and incubated for a certain period of time, so that the fermentation takes place under optimal physiological conditions. Oxygen in the form of air, defoamer, and acid or base are added to control the pH during the fermentation process. During the incubation process, the microorganisms multiply and go through different stages of growth and metabolism; therefore, the composition, biomass, and metabolites of the culture medium change. The development of fed batch fermentation includes the process of feeding a carbon source to the cell culture in a controlled manner [3]. Fed batch fermentation allows the limiting of the substrate of the culture and therefore avoids the generation of by-products that are usually associated with excess residual glucose. The fed batch approach can be divided into two different strategies: fixed volume fed batch, and variable volume fed batch. In the fixed volume scenario, the carbon source is fed without dilution of the culture [4]. In 2019, Anna-Lena Altenhoff, Sven Thierbach, and another researcher used the fed batch fermentation strategy to grow E. coli K30 with VH2 (latex clearing protein) and obtained a cell dry weight of 60 g/L using the pET-23a plasmid and 223 mg/L of soluble active Lcp1VH2, achieving a nearly tenfold increase in yield compared to the fermentation process using the same strain but with a complex autoinduction medium [5].

Modeling for fed batch cultures has also been attempted. In June 2021, a fermentation process based on the construction of a dynamic compartment model to simulate replenishment batches was carried out. This simulation allows for the spatiotemporal characterization of all process variables and the quantification of the metabolic regime experienced by the cells over time [6]. This model uses S. cerevisiae as a reference and applies more than 30 parameters (**Figure 1**) through four phases:

(1) design of CFD-based automatic compartments for different volumes;
(2) construction of a library of compartmental maps for different volumes;
(3) making measurements of the growth caused by the feeding strategy on the original volume;
(4) calculating the total mass transfer coefficient and the oxygen concentration at saturation in each compartment separately.

Nomenclature

Abbreviations

BF	bottom feeding
BMP	bottom monitoring point
CFD	computational fluid dynamics
CM	compartment model
IM	ideal mixing
PI	proportional-integral
TF	top feeding
TMP	top monitoring point

Roman letters

a	interfacial area [m^2 m^{-3}]
BP	by-product concentration [kg m^{-3}]
BP_{Comp}	by-product concentration in each compartment [kg m^{-3}]
$BP_{in,Comp}$	by-product concentration at the inlet in each compartment [kg m^{-3}]
C_{S_i}	linearization coefficient for variable i [s^{-1}]
D	impeller diameter [m]
d_b	bubble diameter [m]
$d_{b,out}$	bubble diameter at the outlet [m]
F_{Bias}	feed rate at the beginning of the fed-batch phase with proportional-integral control [kg h^{-1}]
F_{CO2}	carbon dioxide removal flow rate [kg h^{-1}]
F_{Evap}	evaporation rate [kg h^{-1}]
F_{Exp}	exponential feed rate [kg h^{-1}]
F_{Feed}	glucose solution feeding rate [kg h^{-1}]
F_{in}	inflow in each compartment [kg h^{-1}]
F_{O2}	oxygen transfer flow rate [kg h^{-1}]
F_{out}	outflow in each compartment [kg h^{-1}]
F_{PI}	feed rate when the proportional-integral controller is used [kg h^{-1}]
G	glucose concentration [kg m^{-3}]
G_{Comp}	glucose concentration in each compartment [kg m^{-3}]
G_{Feed}	glucose concentration at the feeding solution [g kg^{-1}]
$G_{in,Comp}$	glucose concentration at the inlet in each compartment [kg m^{-3}]
H	height of the two-phase mixture [m]
H_O	Henry's law constant for oxygen [Pa m^3 kg^{-1}]
I	integral component [kg h^{-1}]
K_{BP}	affinity constant of by-product [kg m^{-3}]
K_c	gain of the proportional-integral component [kg^2 $g^{-1}h^{-1}$]
K_G	affinity constant of glucose [kg m^{-3}]
k_L	liquid film mass transfer coefficient [m s^{-1}]
k_La	overall mass transfer coefficient [h^{-1}]
k_La_{Comp}	overall mass transfer coefficient in each compartment [h^{-1}]
m_{BP}	maintenance coefficient for by-product [kg $kg^{-1}h^{-1}$]
$M_{BP,Comp}$	by-product mass in each compartment [kg]
m_G	maintenance coefficient for glucose [kg $kg^{-1}h^{-1}$]
$M_{G,Comp}$	glucose mass in each compartment [kg]
M_L	liquid mass [kg]
$M_{L,Comp}$	liquid mass in each compartment [kg]
m_O	maintenance coefficient for oxygen [kg $kg^{-1}h^{-1}$]
$M_{O,Comp}$	dissolved oxygen mass in each compartment [kg]
$M_{w,CO2}$	molecular weight of carbon dioxide [g mol^{-1}]
$M_{w,H2O}$	molecular weight of water [g mol^{-1}]
$M_{w,O2}$	molecular weight of oxygen [g mol^{-1}]
M_X	biomass mass [kg]
$M_{X,Comp}$	biomass mass in each compartment [kg]
N	agitation speed [s^{-1}]
O	dissolved oxygen concentration [kg m^{-3}]
O^*	oxygen concentration at saturation [kg m^{-3}]
μ_{BP}	specific growth rate on by-product [h^{-1}]
$\mu_{BP,max}$	maximum specific growth rate on by-product [h^{-1}]
μ_{Comp}	specific growth rate in each compartment [h^{-1}]
μ_{crit}	critical specific growth rate at which overflow metabolism starts [h^{-1}]
O_{Comp}	dissolved oxygen concentration in each compartment [kg m^{-3}]
O^*_{Comp}	oxygen concentration at saturation in each compartment [kg m^{-3}]
$O_{in,Comp}$	dissolved oxygen concentration at the inlet in each compartment [kg m^{-3}]
O_{set}	dissolved oxygen concentration set point [kg m^{-3}]
OTR	oxygen transfer rate [kg $m^{-3}h^{-1}$]
P_{abs}	absolute pressure [Pa]
P_{in}	power input [W]
p^*_{in}	saturated vapour pressure at the inlet [Pa]
Po	Impeller power number [-]
p^*_{out}	saturated vapour pressure at the outlet [Pa]
P_{ref}	reference pressure [Pa]
q_{BP}	specific by-product uptake and formation rate [kg $kg^{-1}h^{-1}$]
$q_{BP,Comp}$	specific by-product uptake and formation rate in each compartment [kg $kg^{-1}h^{-1}$]
q_G	specific glucose uptake rate [kg $kg^{-1}h^{-1}$]
Q_G	air flow rate [NL h^{-1}]
$q_{G,Comp}$	specific glucose uptake rate in each compartment [kg $kg^{-1}h^{-1}$]
$q_{G,crit}$	critical specific glucose uptake rate at which overflow metabolism starts [kg $kg^{-1}h^{-1}$]
q_O	specific oxygen uptake rate [kg $kg^{-1}h^{-1}$]
$q_{O,Comp}$	specific oxygen uptake rate in each compartment [kg $kg^{-1}h^{-1}$]
R	ideal gas law constant [J mol^{-1} K^{-1}]
RH	relative humidity [%]
S_i	source term for production or consumption of variable i [kg m^{-3} s^{-1}]
t	time [h]
T_{Imp}	impeller torque [N m]
T_{in}	temperature at the inlet [K]
T_{out}	temperature at the outlet [K]
t_s	time step size [s]
V_L	liquid volume [m^3]
V_T	total volume [m^3]
V_{T0}	initial total volume [m^3]
X	biomass concentration [kg m^{-3}]
X_{Comp}	biomass concentration in each compartment [kg m^{-3}]
$X_{in,Comp}$	biomass concentration at the inlet in each compartment [kg m^{-3}]
y	mole fraction of oxygen in the gas phase [-]
Y^{Of}_{XBP}	yield coefficient of biomass on by-product under overflow conditions [kg kg^{-1}]
Y^{Ox}_{XBP}	yield coefficient of biomass on by-product under oxidation conditions [kg kg^{-1}]
Y^{Of}_{XG}	yield coefficient of biomass on glucose under overflow conditions [kg kg^{-1}]
Y^{Ox}_{XG}	yield coefficient of biomass on glucose under oxidation conditions [kg kg^{-1}]
Y^{BP}_{XO}	yield coefficient of biomass on oxygen when growth is on by-product [kg kg^{-1}]
Y^{G}_{XO}	yield coefficient of biomass on oxygen when growth is on glucose [kg kg^{-1}]

Greek letters

α	gas volume fraction [-]
β_{k_La}	fraction of the overall mass transfer coefficient at a certain volume with respect to the initial volume [-]
β_{O^*}	fraction of the oxygen concentration at saturation at a certain volume with respect to the initial volume [-]
μ	specific growth rate [h^{-1}]
μ_G	specific growth rate on glucose [h^{-1}]
$\mu_{G,max}$	maximum specific growth rate on glucose [h^{-1}]
μ_{set}	specific growth rate set point [h^{-1}]
τ	time constant of the proportional-integral component [h]

Figure 1. Parameters

The researchers successfully solved the dynamic modeling (see **Table 2** for equations and **Figure 2** for model details).

Table 2. Equations of specific rates for growth (μ), glucose uptake (q_G), ethanol formation, and re-assimilation (q_{BP}) and oxygen uptake (q_O) of the kinetic model used

Regime	Conditions	$\mu[h^{-1}]$	$q_G\ [kgkg^{-1}h^{-1}]$	$q_{BP}[kgkg^{-1}h^{-1}]$	$q_O[kgkg^{-1}h^{-1}]$
Glucose starvation	$\frac{G}{t_s X} < m_G,\ \frac{O}{t_s X} > m_O$	μ_{BP} (8)	$\frac{G}{t_s X}$ (11)	$-\min\left(\frac{\mu_{BP}}{Y^{Ox}_{XBP}} + m_{BP},\ \frac{BP}{t_s X}\right)$ (15)	$-\min\left(\frac{\mu_G}{Y^{G}_{XO}} + \frac{\mu_{BP}}{Y^{BP}_{XO}} + m_O,\ \frac{\mu_{crit}}{Y_{XO}} + m_O\right)$ (19)
Oxidation	$\frac{G}{t_s X} > m_G,\ \frac{O}{t_s X} > m_O,\ q_G < q_{G,crit}$	$\mu_G + \mu_{BP}$ (9)	$-\left(\frac{\mu_G}{Y^{Ox}_{XG}} + m_G\right)$ (12)	$-\frac{\mu_{BP}}{Y^{Ox}_{XBP}}$ (16)	
Overflow	$\frac{O}{t_s X} > m_O,\ q_G > q_{G,crit}$	μ_G (10)	$-\left(\frac{\mu_{crit}}{Y^{Ox}_{XG}} + \frac{\mu_G - \mu_{crit}}{Y^{Of}_{XG}} + m_G\right)$ (13)	$\frac{\mu_G - \mu_{crit}}{Y^{Of}_{XBP}}$ (17)	
Oxygen limitation	$\frac{G}{t_s X} > m_G,\ \frac{O}{t_s X} < m_O$		$-\left(\frac{\mu_G}{Y^{Of}_{XG}} + m_G\right)$ (14)	$\frac{\mu_G}{Y^{Of}_{XBP}}$ (18)	$-\frac{O}{t_s X}$ (20)
Glucose starvation and oxygen limitation	$\frac{G}{t_s X} < m_G,\ \frac{O}{t_s X} < m_O$	n.a.	$-\frac{G}{t_s X}$ (10)	n.a.	

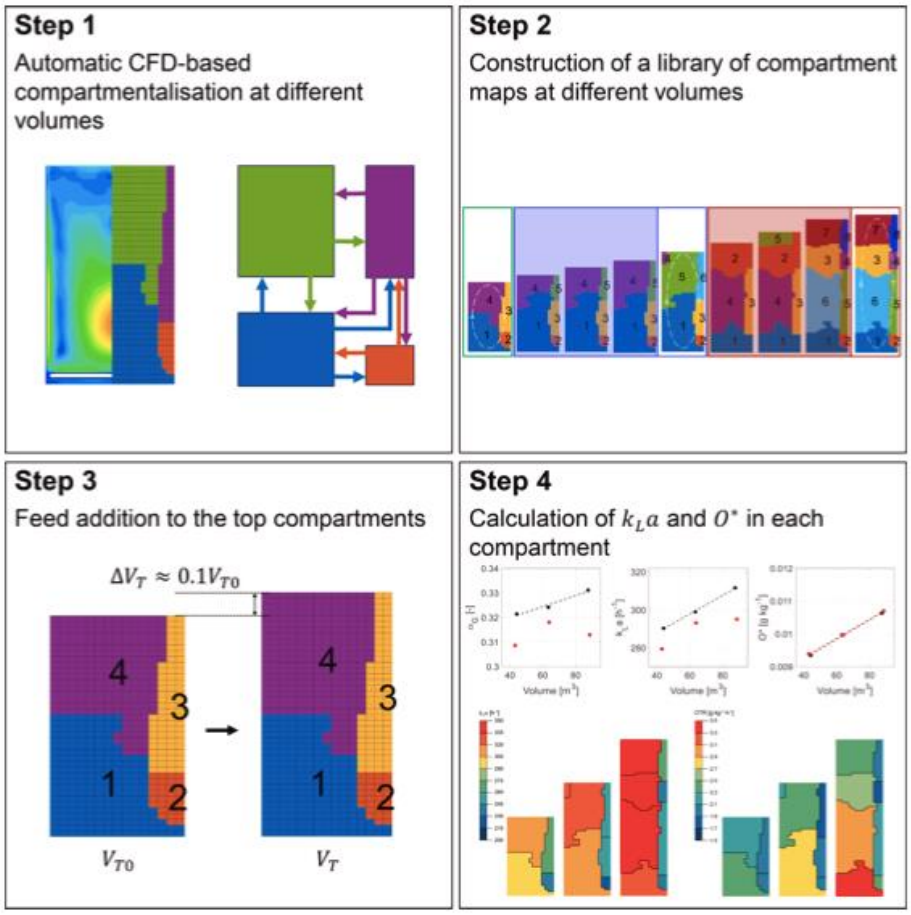

Figure 2. Schematic showing the methodology used to set-up and solve the dynamic compartment model

2.2. Continuous fermentation

Continuous fermentation also utilizes a closed fermentation system. This fermentation system can operate without limits by continuously or intermittently adding fresh nutrient media to the fermenter. As new medium is added, the system continuously pumps an equal amount of old medium with microorganisms for fermentation product processing and the recovery of cells [3]. This fermentation system theoretically maintains optimal volume and nutrient concentrations as well as reduces downtime and operating costs.

In 1981, Hughes and Richardson patented a fermentation process from their experiments for the continuous production of polyhydroxybutyrate (PHB) using Alcaligenes sp. in a single fermenter.

Continuous fermentation of Alcaligenes sp. was achieved by providing a continuous medium containing nutrient salts, carbon and energy sources, as well as water-soluble compounds assimilated by the microorganisms. They also designed the fermentation system to remove an equal amount of the medium containing the bacterial cells from the medium, thus keeping the amount of aqueous medium in the vessel constant. Hughes and Richardson's invention can be regarded as the founding of continuous fermentation.

In recent years, the usage of continuous fermentation has become more and more widespread. For example, Imperial Chemical Industries (ICI), a British company, uses continuous culture for the production of single-cell proteins in giant fermenters with a volume of 1,500 m^3, achieving an annual production of 70,000 tons. Meanwhile, several researchers have successfully increased ethanol production with continuous fermentation in a two-tank system [7]. Continuous fermentation has also been applied in agriculture; using dehulled rice as raw material, a scientific team led by Guoqing Wu designed a semi-continuous fermentation system in the laboratory (**Figure 3**), resulting in an average alcohol content of 15.36% per volume of the mature brown rice fermented mash [8].

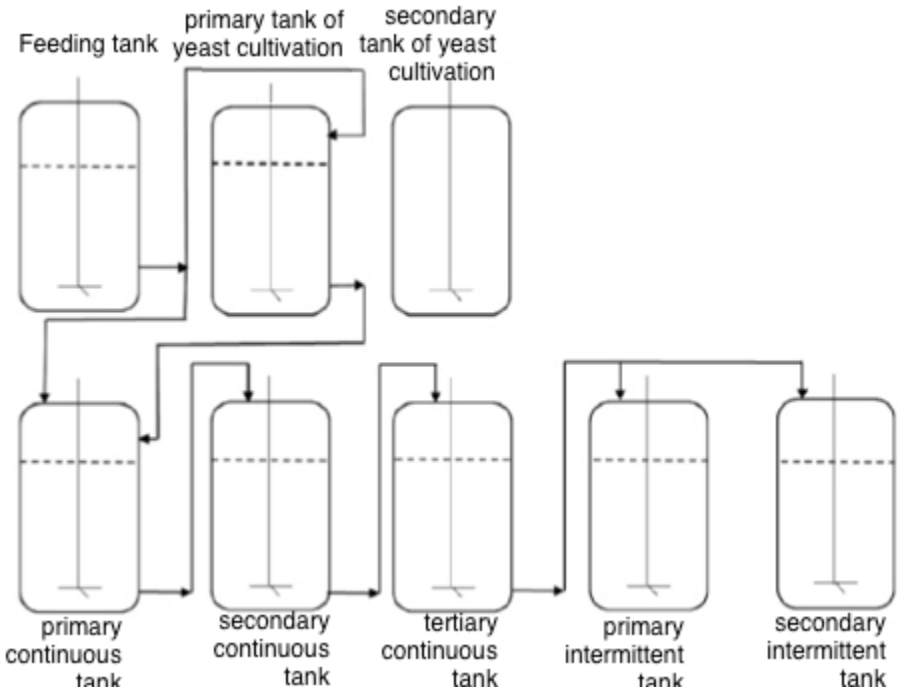

Figure 3. Diagram showing the semi-continuous fermentation system

Disadvantages do exist. The contamination by debris and the strain genetics are not well controlled. Furthermore, continuous fermentation has not been commonly used in industries for the past two decades to produce large amounts of microbial metabolites.

3. Summary

In addition to these typical methods, there are also different industrial fermentation methods, such as submerged fermentation and aseptic fermentation. The scientific and commercial communities are constantly developing and adapting different fermentation processes according to their own needs and those of the society. In the near future, the development of fermentation processes will continue to hit new heights of efficiency and yield.

Disclosure statement

The author declares no conflict of interest.

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