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Unstructured Modeling of Aureobasidium Pullulans Fermentation for Pullulan Production- a Mathematical Approach

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***Abstract*- Mathematical Biology gained much attention over the past few decades as mathematical modeling, creates large models with the pivotal factors, which was quite successful in industrial fermentations. A detailed, unstructured, mathematical kinetic model is presented here for both the batch and continuous production of eco-friendly biopolymer, Pullulan, from *Aureobasidium pullulans*, which uses variety of agro-industrial wastes. Set of ordinary differential equations were developed as Logistic (*L*) model for *A. pullulans* growth, Logistic Iincorporated Leudeking-Piret (*LILP*) model for Pullulan formation and Logistic Incorporated Modified Leudeking-Piret (*LIMLP*) model for various substrates utilization, to understand the dynamic behavior of batch fermentation. Next, the similar set of equations for a steady-state, continuous system of Pullulan fermentation was also developed. These models would offer more insights of Pullulan optimization with better productivities.**

Keywords: Pullulan; unstructured kinetics;L;LILP;LIMLP models;agro-industrial wastes.

I. INTRODUCTION

In the recent years, the renewed interest in the scientific community is towards quest for production of polymers from sustainable and eco-friendly benign sources like plants or organic waste. The biopolymers derived from these renewable sources, not only provide alternatives to the synthetic polymers, but also get degraded into environment very quickly[6]. One of such industrially renowned

For this purpose, the fermenter design has, always, to be combined with mathematical modeling of fermentation process in simpler and quite effective way[4, 13]. Usually, the unstructured mathematical models that account biomass as a single variable have fully proven to represent the biomass growth, substrate utilization and product formation[3, 7, 16 19]. Structured models, in contrast, consider the changes in biomass composition that significantly influence the kinetics part. Logistic model has been used to represent the growth of *A. pullulans*and estimated the biomass productivity at various sucrose concentrations [14, 15]. In addition, non-growth associated Pullulan formation takes place, in the late exponential phase, i.e., as a secondary metabolite[26].A similar kind of mathematical model is studied to explain the kinetic-metabolic nature of Pullulan production.

biopolymer, Pullulan, shows broader range of applications in food, pharma, cosmetic and other sectors.

Pullulan, an ExoPolySaccharide (EPS), secreted by a black yeast-like fungus, *Aureobasidium pullulans* (previously known as *Pullularia pullulans*), consists of α–maltotriose units arranged in linear 1-6 linkage on the terminal trisaccharide glucose residue. Due to its less Oxygen permeability, Pullulan biopolymer allows the great potential use in food packaging films, coating of food containers for perishable fruit and vegetables, and obtained the status of "Generally Regarded As Safe' (GRAS) by USFDA[22]. The other demonstrated applications of Pullulan lies in biomedical field for drug and gene delivery, tissue engineering, wound healing and quantum dots in diagnostic imaging [17]. Though the tremendous practical applicability of Pullulan was investigated by many researchers, the use of Pullulan for the production of biodegradable plastic is constrained by its selling price ($25/Kg.)[21]. Despite, Pullulan could be produced through fermentation using cheaper substrates; the current focus of research is on molecular and genetic modifications in the producer strain and operative strategies for higher Pullulan yields [20].

Commercial success of any industrial fermentation, especially in expensive biomaterials production, depends on maintenance of controlled conditions in complex biological reactions.

Hence, the developed model, presented here in this work, is divided into two parts: the kinetic modelsfor*Aureobasidium pullulans* growth, Substrate consumption andPullulan formation in a batch systemare reviewed in first part and followed by the extension of batch kinetic models for continuous culture system to characterize the variations in Pullulan fermentation, in the second part.

II. MATHEMATICAL MODELS

A. Modeling of Pullulan fermentation in a batch system

a) Kinetic Modeling of *Aureobasidium pullulans*(biomass) growth

Assuming negligible substrate and product inhibitory effects, under optimal growth conditions,a simplified growth model of *A. pullulans* (X), according to Malthus's Population theory, under batch fermentation kinetics can be given as[12, 23, 24]:

$$\frac{dX}{dt} = \mu_{max} X \left(1 - \frac{X}{X_m}\right) \quad (1)$$

whereX_m is the maximum attainable *A. pullulans* concentration.

The integrated form of above equation results in the following Logistic (L) model equation:

$$X_t = \frac{X_0 e^{\mu max\ t}}{1-\frac{X_0}{X_m}(1-e^{\mu max\ t})} \quad (2)$$

Logistic model expression, equation (2), neglects the effect of substrate concentration on the biomass growth rate and when X_t approaching X_m, the specific biomass growth rate (μ_{max}) approaches to zero.

b) Kinetic Modeling of Pullulan (product) production

Pullulan formation rate, from Luedeking-Piret [11] kinetics (i.e., a two-parameter model), shows the linear dependency on *A. pullulans* concentration (instant) and growth rate, as:

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \quad (3)$$

where α is a constant obtained in product formation during growth of *A.pullulans* and β is product formation activity/ mass of cells.The values of α and β are empirical constants that may vary with fermentation environmental conditions like temperature, pH and agitation and aeration rates.

To relate the Pullulan formation with time, equation (3) is solved by substitution of equations (1) & (2) in to Eq. (3) yields:

$$P_t = P_0 + \alpha \left[\frac{X_0 e^{\mu max\ t}}{1-\left(\frac{X_0}{X_m}\right)(1-e^{\mu max\ t})} - X_0\right] + \frac{\beta X_m}{\mu} ln\left[1 - \left(\frac{X_0}{X_m}\right)(1 - e^{\mu_{max}\ t})\right] \quad (4)$$

This Logistic incorporated Luedeking-Piret (LILP) kinetic expression (Eq. (4)) was used in fitting data for many biopolymers production [10, 24, 25].

As β is non-growth associated(not depends on growth phase) parameter, the value of β has to be evaluated with the help of stationary phase data (where $\frac{dX}{dt} = 0$):

$$\beta = \frac{\left(\frac{dP}{dt}\right)_{stationary\ \ phase}}{X_{max}} \quad (5)$$

During initial phases of any fermentation (even in this case), the concentration of product in the media is absolutely zero, i.e., P_0=0. So, the value of α can be determined using equation (4).

c)Kinetic Modeling of Sucrose (substrate) consumption

Although, *Aureobasidium pullulans* can grow on variety of carbon substrates, the better yields of Pullulan were made possible with sucrose as a sole carbon source.*Aureobasidium pullulans* utilizes the available sucrose for supporting the cell viability (even in absence of growth), cell motility, osmotic functions, enzyme turnover, and nutrient storage and also for otherworks referred as maintenance activity. So, sucrose (S) consumption rate can be summed up with sucrose utilization for *A.pullulans*'s growth and above mentioned maintenance activity, and for Pullulan formation, as follows:

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}}\left(\frac{dX}{dt}\right) - \frac{1}{Y_{P/S}}\left(\frac{dP}{dt}\right) + m_S X \quad (6)$$

where$Y_{X/S}$ and $Y_{P/S}$ are Yield coefficients of cell and product on substrate, and m_S is the maintenance energy coefficient, h^{-1}.

The equation (6) can be rearranged to represent as Modified Leudeking-Piret (MLP)equation[15]:

$$-\frac{dS}{dt} = r_S = \gamma\left(\frac{dX}{dt}\right) + \eta X \quad (7)$$

To find out the Sucrose consumption variation with time, equation (7) upon integration and by substituting the equations (1) & (2) in Eq. (7), results a Logistic incorporated Modified Leudeking-Piret (LIMLP) equation:

$$S_t = S_0 - \gamma\left[\frac{X_0 e^{\mu max\ t}}{1-\left(\frac{X_0}{X_m}\right)(1-e^{\mu max\ t})} - X_0\right] + \frac{\eta X_m}{\mu} ln\left[1 - \left(\frac{X_0}{X_m}\right)(1 - e^{\mu_{max}\ t})\right] \quad (8)$$

Similar toproduct formation kinetics, nongrowth-associated constant, η, in substrate utilization kineticsis also calculated from stationary phase data:

$$\eta = \frac{-\left(\frac{dS}{dt}\right)_{stationary\ \ phase}}{X_{max}} \quad (9)$$

And, a growth-associated constant, γ, in substrate utilization kineticscan be determined using equation (8).

Thus, equations (2), (4) & (8) can be solved simultaneously, using sufficient kinetic parameters to study the behavior of *Aureobasidium pullulans* growth, Pullulan formation and Sucrose consumption, under batch fermentation conditions.

B. Modeling of Pullulan fermentation in a Continuous system

Description: Consider a submerged continuous fermentation system, as shown in figure 1, in which the input concentrations of Sucrose, *A.pullulans* and Pullulan, as S_0, X_0 and P_0, respectively, enters the fermenter at a constant volumetric feed flow rate, *F*. The output concentrations of *S*, *X* and *P* for Sucrose, *A.pullulans* and Pullulan, respectively, exit from the system with same output flow rate, *F*.

d) Kinetic Modeling of A.pullulans growth

Idealized fermentation kinetics, depend on transport of nutrients to the cell surface, the mass transfer rate from medium into cells and also environmental conditions (temperature, pH, etc), which are maintained at optimal level [18].

The batch fermentation model, explained previously, for kinetics of Pullulan production has used the Logistic equation to represent the *A.pullulans* growth, can be arranged (from equation (1)) as follows:

$$\mu = \mu_{max}\left(1 - \frac{X}{X_m}\right) \quad (10)$$

where$\mu$and μ_{max} are specific and maximum specific growth rates of *A.pullulans*, h^{-1}.

Applying Law of mass conservation for the components of continuous fermentation, as in the following form:

Accumulation rate = Input rate – Output rate + Generation rate – Consumption rate (11)

For *A.pullulans* (*X*) component

$$\frac{dX}{dt}(V) = FX_0 - FX + \mu XV \quad (12)$$

On substitution of equation (10),

$$\frac{dX}{dt}(V) = D(X_0 - X) + \mu_{max}\left(1 - \frac{X}{X_m}\right)X \quad (13)$$

Where *D* is dilution rate, h^{-1}, is defined as $D = F/V$.

Assume that steady-state prevails inside continuous system and sterile feed ($X_0 \approx 0$) enters the fermenter, the equation (13) is modified as:

$$D = \mu = \mu_{max}\left(1 - \frac{X}{X_m}\right) \quad (14)$$

e) Kinetic Modeling of Sucrose consumption

For Sucrose (*S*) component

By applying the mass balance, equation (11) for the key substrate, Sucrose (S) component yields as:

$$\frac{dS}{dt}(V) = FS_0 - FS - r_S V \quad (15)$$

Where $r_S$is the rate of Sucrose consumption, g/(L.h).

The assumption of steady-state condition in the fermenter and substitution of Logistic equation (7) results an altered expression of equation (15), as:

$$(S_0 - S) = X\left[\gamma + \frac{\eta}{\mu_{max}\left(1-\frac{X}{X_m}\right)}\right] \quad (16)$$

f) Kinetic Modeling of Pullulan production

For Pullulan (*P*) production

Lastly, the mass balance for Pullulan produced in the fermentation can be derived as:

$$\frac{dP}{dt}(V) = FP_0 - FP + r_P V \quad (17)$$

Where $r_P$is the rate of Sucrose consumption, g/(L.h).

Assuming steady-state behavior and product-free stream enters the continuous system; equation (17) is rearranged by substituting Luedeking-Piret equation (3):

$$P = X\left[\alpha + \frac{\beta}{\mu_{max}\left(1-\frac{X}{X_m}\right)}\right] \quad (18)$$

Thus, the equations (14), (16) & (18) can be solved simultaneously to study the profiles of concentrations of *Aureobasidium pullulans*, Sucrose and Pullulan performances, respectively, under steady-state behavior in continuous fermentation system.

Mathematical analysis of any fermentation data, can convincingly explain the kinetics of biomass, substrate and product with unstructured models [1, 2]. In general, these models give us good approximation of parameter profiles even though we do not consider the complete mechanism of microbial growth. Table 1 had shown estimated parameters for Pullulan production by *Aureobasidium pullulans* spp. grown on various agro-industrial wastes in batch mode using unstructured models.

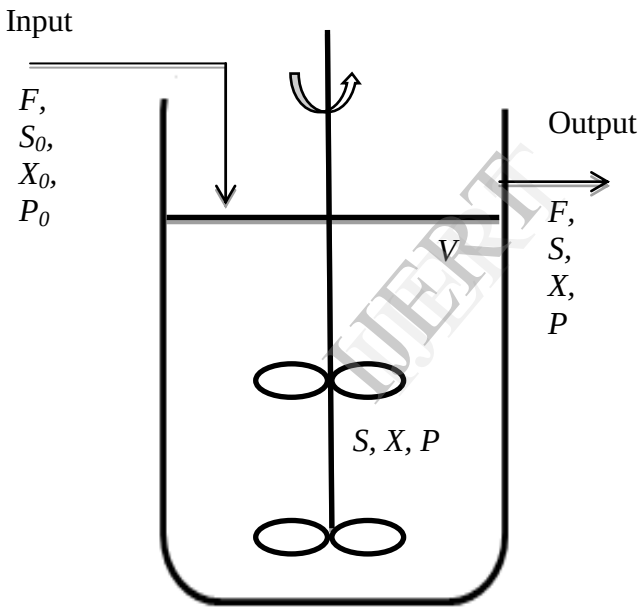

Figure-1.Schematic representation of Continuous fermentation system.

In its first ever report on unstructured modeling of Pullulan fermentation, Mohammad et al.,have also performed modeling studies by *Aureobasidium pullulans* III for increasing sucrose concentrations in batch kinetics [14]. In a batch fermentative production of Pullulan from *Aureobasidium pullulans* from low cost substrate like cashew fruit juice, K. Thirumavalavan*et al*., concluded that the kinetic models used by them did reasonably fit between the theoretical and experimental data [8]. K.C. Cheng *et al*., reported that the Re-modified Leudeking-Piret model (after taking into account the cessation of biomass production) clearly represented the Sucrose utilization with good R^2 (≈0.995) value, in the Pullulan fermentation by a colour variant strain of *A. pullulans* [9].

III. CONCLUSION

In this study, Mathematical modeling approaches of batch and continuous fermentation of *Aureobasidium pullulans* for Pullulan production were developed. These primary models of *A. pullulans* growth, Pullulan production and Sucrose consumption in a batch system, were successfully proven to be in good agreement with the experimental results, as indicated by many researchers. But, a detailed model on Pullulan biopolymer production in a continuous system was not reported, till date. Hence, the models presented here could be used for the simulation of

optimal parameters that match the real-time Pullulan fermentation.

Table-1.Estimated kinetic parameters used in modeling of Pullulan production from *Aureobasidium pullulans* spp. on different carbon substrates in batch fermentation.

Substrate (*S*) used		L model parameters			LILPmodel parameters		LIMLP model parameters		Reference
Type	S_0 (g/l)	μ_{max} (h^{-1})	X_0 (g/l)	X_m (g/l)	$\alpha^{\dagger}$	$\beta^{\ddagger}$	$\gamma^{\shortparallel}$	η^{*}	
Glucose	50	--	1.0	--	1.925	0.04	1.4	0.08	[5]
Sucrose	50	--	1.0	--	0.383	0.04	9.4	0.2	
Beet Molasses	100	--	1.0	--	0.265	0.04	12.88	0.27	
Sucrose	25	0.035	0.161	0.501	4.75	0.0092	3.67	0.0008	[14]
	50	0.042	0.11	0.792	7.69	0.01	3.16	0.009	
	100	0.02	0.142	0.923	8.89	0.0204	4.8	0..0168	
	200	0.023	0.151	0.721	7.14	0.066	5.6	0.064	
Sucrose	75.8	0.048	0.8	28.3	0.79	0.0047	2.61	0.007	[9]
Cashew fruit juice	50	0.07	1.0	92.0	0.9	0.001	0.98	0.001	[8]

†- g product/ g biomass; ‡- g product/(g biomass h); $^{\shortparallel}$- g substrate/ g biomass; *- g substrate/(g biomass h)
L- Logistic, LILP- Logistic incorporated Leudeking-Piret, LIMLP- Logistic incorporated Modified Leudeking-Piret

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