British Biotechnology Journal
9(3): 1-14, 2015, Article no.BBJ.20476
ISSN: 2231–2927

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Mathematical Modeling of Macroscale Phenomena: Oxygen Transfer for Solid-state Fermentation in Static Tray Bioreactor

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Authors' contributions

Authors JSMP, TB and SNG designed the study. Author JSMP performed the simulation studies wrote the first draft of the manuscript. Authors SNG and TB revised the manuscript critically. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BBJ/2015/20476
Editor(s):
(1) Anil Kumar, School of Biotechnology, Devi Ahilya University, Madhya Pradesh, India.
Reviewers:
(1) Gyula Oros, Plant Protection Institute of the Centre for Agricultural Research of the Hungarian Academy of Sciences, Budapest, Hungary.
(2) Anonymous, Gautam Buddha University, Greater Noida, India.
Complete Peer review History: http://sciencedomain.org/review-history/11229

Original Research Article

Received 29th July 2015
Accepted 23rd August 2015
Published 2nd September 2015

ABSTRACT

Aims: To develop a mathematical model for prediction of variation in oxygen concentration inside the bed of tray type bioreactor for solid state fermentation and comparison of oxygen profile of unsteady state and pseudo steady state approximation.
Place and Duration of Study: All the simulations were performed at the Applied and Industrial Microbiology laboratory, Indian Institute of Technology, Madras, from October 2013 to September 2014.
Methodology: Models for various reaction kinetics (zero order, first order and Monod's kinetics) were derived from the general model. Ordinary differential equations (Pseudo steady state approximation) and partial differential equations (unsteady state assumption) were solved by numerical techniques – Finite difference method (FDM) and Runge-kutta method. Simulation runs were carried out for various parameters such as bed height gas phase oxygen concentration, saturation constant, and porosity of the bed.

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Results: Oxygen profiles of unsteady state and pseudo steady state assumption were compared and results show lower oxygen concentration in case of unsteady state assumption. Concentration of oxygen was low for the organism following first order when compared to zero order and Monod kinetics. Results of simulation runs revealed that the oxygen concentration decreases as the bed height increases irrespective of the kinetics of the reaction. And it increases with increasing gas phase oxygen concentration, saturation constant and porosity.
Conclusion: Mathematical model with unsteady state assumption was reported and it can be employed in calculating the design and operational parameters for solid state fermentors to yield optimal productivity.

Keywords: Solid-state fermentation; oxygen transfer; bioreactor; modeling; kinetic parameters; Finite difference method.

NOMENCLATURES

X	*Biomass concentration (g cm^{-3})*	X_{max}	*Maximum biomass concentration (g cm^{-3})*
μ_{max}	*Maximum specific growth rate (h^{-1})*	R	*Reaction rate (g cm^{-3} h^{-1})*
C	*Oxygen concentration in the bed (g cm^{-3})*	C_{O_2}	*Gas phase oxygen concentration (g cm^{-3})*
D_e	*Effective diffusivity (cm^2 s^{-1})*	z	*Height at which rate is measured (cm)*
K_s	*Substrate saturation constant (g cm^{-3})*	ε	*Porosity of the solid bed*
t	*Fermentation time (h)*	$Y_{X/S}$	*Yield coefficient (g g^{-1})*
H	*Total height of the solid bed (cm)*	z^*	*Dimensionless tray depth*
C^*	*Dimensionless oxygen concentration*	t^*	*Dimensionless fermentation time*
β	*Dimensionless substrate saturation constant*	Φ_0	*Thiele modulus for zero order reaction*
Φ_1	*Thiele modulus for first order reaction*	Φ_M	*Thiele modulus for Monod kinetics*

GREEK SYMBOLS

β	*Dimensionless substrate saturation constant*	ε	*Porosity of the solid bed*
μ	*Specific growth rate (h^{-1})*	Φ	*Thiele modulus*

1. INTRODUCTION

Solid state fermentation (SSF), involves the growth of microorganisms on solid substrate in the absence of free moving water [1]. SSF has been successfully exploited for bioremediation and biodegradation of hazardous compounds, biological detoxification of toxic agro-industrial residues, biotransformation of crops and crop residues for nutritional enrichment, biopulping, etc. Biologically active secondary metabolites, including antibiotics and drugs, enzymes, organic acids, biopesticides, biofuel, biosurfactants and food flavor compounds were produced by SSF process [2]. SSF extends many advantages over submerged fermentation (SmF) such as higher production yields [3], lower capital costs and operating costs, low energy requirements, low investment outlays, higher volumetric yield [4]. In addition, simpler downstream processing produces less waste water than SmF and is environmental friendly as it resolves the problem of solid waste disposal by utilizing cheaper substrates such as agricultural solid wastes [5].

Nevertheless, SSF processes are slower than SmF due to the additional barrier from the bulk solid. Absence of free water in the substrate bed leads to poor heat removal characteristics and difficulty in agitation of the substrate bed due to high viscous solids resulting in heterogeneously distributed physiological, physical and chemical environment in the substrate bed [6]. Heat dissipation problems can be limited by interparticle as well as intraparticle resistances and they are more difficult to control due to the lack of adequate sensors and efficient solid handling techniques [7]. Difficulty in fermentation control, heat build-up, trouble in controlling the moisture level of the substrate and aeration,

difficulty in rapid determination of microbial growth and other fermentative parameters and limited types of microorganisms which can grow at low moisture levels are some of the shortcomings of SSF which renders its industrial application [8].

Solid substrate fermentation on a large scale usually results in unsatisfactory yield. Bed heterogeneity in large scale SSF bioreactor is unavoidable primarily due to low heat and mass transfer rate characteristics of the solid substrate bed [9]. Scaling up of SSF processes are challenging and unreliable and this can be attributed to the profoundly different growing conditions of microorganisms in large scale reactor [10].

SSF bioreactor performance was controlled by microscale and macroscale phenomena. Microscale phenomena include growth and death rates in response to the environmental conditions, effect of microbial growth on environment through the release of enzymes and end products, intraparticle diffusion of compounds such as CO_2, O_2, nutrients, enzymes and transfer between the interparticle regions. Macroscale phenomena include bulk flow of air into and out of the bioreactor, conduction across the bioreactor wall and convective cooling to the surroundings [6].

Among several macroscale phenomena, transfer of oxygen becomes an important step since the biochemical reaction rates are controlled by the oxygen transfer from the gas phase [11]. Oxygen is considered an essential substrate for fungal growth and secondary metabolite production [12]. In tray type bioreactor with non-perforated bottom, the lack of oxygen at the bottom of the bed limited the growth rate in this region [13]. Limitation in oxygen transfer influences SSF performance. It is essential to predict the optimal operational and design parameters to ensure no zero oxygen concentration zone and proper heat transfer [14].

The concentration gradient which results in heterogeneous condition in the bioreactor can be eliminated by developing a conceptual model [13]. Monitoring and controlling environmental factors, biomass and metabolite production in SSF pose problems which correspond to the intricacy and heterogeneity of the media [15]. Understanding the interaction between fungal growth and physical phenomena is complex in absence of mathematical models. Modeling could be a good tool for designing bioreactors, scale-up and control studies. Mathematical models of SSF will lead to better understanding of the transport processes and assists in designing of bioreactor [8].

Models for oxygen concentration profile were developed by some researchers for zero and first order kinetics with pseudo steady state assumption. Raghavarao and coworkers modelled oxygen concentration profile inside the substrate bed based on the assumption that the biochemical reaction follows zero order kinetics with pseudo steady state assumption [16]. However, in practice, all microorganisms do not follow zero order kinetics. Rajagopalan and Modak [17], considered the fungal growth on the substrate as a biofilm of unicellular organism and it was assumed that oxygen diffuses with the diffusivity of water. And they modelled the film phase oxygen concentration for zero and first order oxygen consumption kinetics. Model for oxygen concentration in the substrate bed with unsteady state assumption was developed by Muniswaran and coworkers [12]. Their model describes the bed oxygen concentration profile for zero order kinetics of oxygen consumption and Malthus equation [12] was considered for biomass growth. In this work, a model has been developed to describe the oxygen concentration prevailing in the bed for organism following zero, first and Monod kinetics [17] in addition to Logistic equation for growth. This model was solved for both pseudo steady state and unsteady state assumptions. This model was adequate to explain the macroscale phenomenon of oxygen transfer inside the static tray bioreactor, which in turn helps in computing the design and operating parameters of tray bioreactor.

Current study aims in the development of mechanistic model to describe the oxygen concentration profile inside the bed of tray bioreactor and to study the variation of oxygen concentration for different reaction kinetics such as zero order, first order and Monod kinetics with unsteady state assumption. Simulation of this model has been carried out with the experimental data of Sugama and Okazaki [18], where the growth of *Aspergillus oryzae* cultured on solid media was estimated. This work also intends to establish the relation between oxygen concentration and varying model parameters such as bed height (H), gas phase oxygen concentration (C_{O_2}), saturation constant (K_s) and bed porosity (ε). A dimensionless modulus, thiele

modulus has been derived, for each reaction kinetics, to explain further control mechanism of the process.

2. METHODOLOGY

2.1 Process Description

Tray type bioreactors are reactors with unmixed beds without forced aeration, suitable for organisms where agitation results in deleterious effects. In this reactor, a number of trays containing thin layer of substrate are enclosed in a chamber into which air is circulated with controlled temperature and relative humidity. Oxygen concentration profile was modelled for tray reactors with open top and unperforated bottom ensuring transfer of oxygen only through diffusion of air and not by forced convection as depicted in Fig.1.

The transport phenomena in a tray type bioreactor involves sequence of process as follows:

1. Transfer of gaseous oxygen from the bulk air to the tray
2. Diffusion of oxygen through interparticle spaces
3. Exchange of oxygen and water between interparticle spaces and biofilm
4. Diffusion of oxygen within the biofilm
5. Biochemical reaction involves the growth of organism based on substrate and oxygen consumption
6. Exchange of carbon dioxide and water between interparticle spaces and biofilm
7. Diffusion of carbon dioxide through interparticle spaces
8. Transfer of carbon dioxide and generated metabolic heat from the tray to the surrounding

2.2 Model Development

2.2.1 Model Assumptions

1. Substrate bed is isothermal. Infinitesimal change in temperature during fermentation is assumed and the reaction rate is constant not to be influenced by change in temperature.
2. Energy source is present in excess and that does not impede the biochemical reaction.
3. Gaseous phase oxygen in the interspatial voids of the bed is considered as the limiting reactant. Growth of organism depends on the oxygen concentration existing in the bed.
4. The kinetics of the growth of organism follows Logistic equation. Mazaheri and Shjaoosadati [19] showed that many fungi follows logistic kinetics. This equation well explains the lag, growth and stationary phases.
5. Rate of consumption of oxygen follows Monod kinetics and is directly proportional to the growth rate of organism.
6. Consumption of oxygen depends on the concentration of oxygen prevailing in the bed

   Case 1: The reaction rate follows first order kinetics if the concentration of oxygen is very low (C_{O_2})<< Ks

   Case 2: The reaction rate follows zero order kinetics if the concentration of oxygen is high (C_{O_2})>> Ks

7. Transfer of oxygen from the bulk air to the tray occurs only through diffusion and there is no convective mass transfer.
8. No mass transfer resistance in the static film gas on the substrate bed.
9. Model parameters remain constant throughout the fermentation.

2.2.2 Growth kinetics

Logistic equation has been used to describe the growth of organism [19]. It is an unstructured, empirical model that gives adequate information about the lag, growth and stationary phases of growth curve.

$$\frac{dX}{dt} = \mu_{\max} X\left(1 - \frac{X}{X_{\max}}\right) = R \ , \qquad (1)$$

where μ_{max} is the maximum specific growth rate (h^{-1}), X is the biomass concentration (mg cm^{-3}) and X_{max} is the maximum biomass concentration (mg cm^{-3}).

2.2.3 Oxygen balance

Oxygen diffuses from the bulk gas over the tray, on to the substrate particle and is consumed by the microorganisms to yield product (Fig.1). Mass balance for gaseous phase oxygen for infinitesimal slab of thickness Δz is given by Eq. 2 as follows:

$$\varepsilon \frac{\partial C}{\partial t} = D_e \frac{\partial^2 C}{\partial z^2} - \frac{RC}{(K_S + C) Y_{X/S}}, \quad (2)$$

Case I: If oxygen concentration is very low (C_{O_2})<<Ks Eq. 2 becomes

$$\varepsilon \frac{\partial C}{\partial t} = D_e \frac{\partial^2 C}{\partial z^2} - \frac{RC}{(K_S) Y_{X/S}}, \quad (3)$$

Case II: If oxygen concentration is very high (C_{O_2})>>Ks Eq. 2 becomes

$$\varepsilon \frac{\partial C}{\partial t} = D_e \frac{\partial^2 C}{\partial z^2} - \frac{R}{Y_{X/S}}, \quad (4)$$

where D_e is the effective diffusivity ($cm^2\ s^{-1}$), z is the infinitesimal height of the substrate bed (cm), C is the gas phase oxygen concentration ($g\ cm^{-3}$), K_s is the substrate saturation constant ($g\ cm^{-3}$), $Y_{X/S}$ is the biomass yield coefficient and ε is the porosity of the substrate bed.

The equations are subject to the following initial and boundary conditions:

$$C = C_{O_2};\ t = 0;\ \forall\ z,$$
$$C = C_{O_2};\ z = 0;\ \forall\ t,$$
$$\partial C/\partial z = 0; z = H; \forall t, \quad (5)$$

The governing equations of oxygen concentration profile for static tray bioreactor (Fig.1) are as follows,

$$\varepsilon \frac{\partial C^*}{\partial t^*} = \frac{\partial^2 C^*}{\partial z^{*2}} - \frac{H^2}{D_e C_{O_2} Y_{X/S}} \frac{RC^*}{(\beta + C^*)}, \quad (6)$$

$$\varepsilon \frac{\partial C^*}{\partial t^*} = \frac{\partial^2 C^*}{\partial z^{*2}} - \frac{H^2}{D_e C_{O_2} Y_{X/S}} \frac{RC^*}{(\beta)}, \quad (7)$$

$$\varepsilon \frac{\partial C^*}{\partial t^*} = \frac{\partial^2 C^*}{\partial z^{*2}} - \frac{RH^2}{D_e C_{O_2} Y_{X/S}}, \quad (8)$$

Using the following dimensionless variables,

$$C^* = C/C_{O_2};\ z^* = z/H;$$
$$t^* = tD_e/H^2;\ \beta = Ks/C_{O_2}; \quad (9)$$

The governing equations [Eqs. 6, 7 and 8] are subject to the following conditions.

$$C^* = 1;\ t^* = 0;\ \forall\ z^*,$$
$$C^* = 1;\ z^* = 0;\ \forall\ t^*,$$
$$\partial C^*/\partial z^* = 0; z^* = 1, \forall t^*; \quad (10)$$

Note that C* and t* (see Eqs. 6 - 8) are the dimensionless oxygen concentration and dimensionless fermentation time, H is the vertical depth of the solid substrate bed and β refers to the dimensionless saturation constant.

2.2.4 Pseudo steady state approximation

Variation of oxygen concentration with respect to time is negligible when compared to the change in oxygen concentration with regard to the tray depth. It is assumed that the spatial profiles of concentration are established much faster than the time profiles [17]. Based on pseudo steady state approximation, Eqs. 6 - 8 the partial differential equations are reduced to second order ordinary differential equations (ODE):

$$\frac{d^2 C^*}{dz^{*2}} - \frac{H^2}{D_e C_{O_2} Y_{X/S}} \frac{RC^*}{(\beta + C^*)} = 0; \quad (11)$$

$$\frac{d^2 C^*}{dz^{*2}} - \frac{H^2}{D_e C_{O_2} Y_{X/S}} \frac{RC^*}{(\beta)} = 0; \quad (12)$$

$$\frac{d^2 C^*}{dz^{*2}} - \frac{RH^2}{D_e C_{O_2} Y_{X/S}} = 0; \quad (13)$$

Eqs. 11, 12 and 13 are subject to the following boundary conditions.

$$C^* = 1,\ z^* = 0;$$
$$dC^*/dz^* = 0,\ z^* = 1; \quad (14)$$

2.3 Numerical Simulation, Validation and Parameters

Ordinary differential equations (ODEs) (Eqs. 11, 12, 13) with the boundary conditions are solved using fourth-order Runge-Kutta method with variable space step size defined by the function ode45 in MATLAB - (Version 9.0). The partial derivatives in Eqs. 6, 7, 8 are discretized by finite difference method using central difference approximation with equal grids and solved simultaneously in MATLAB [20].

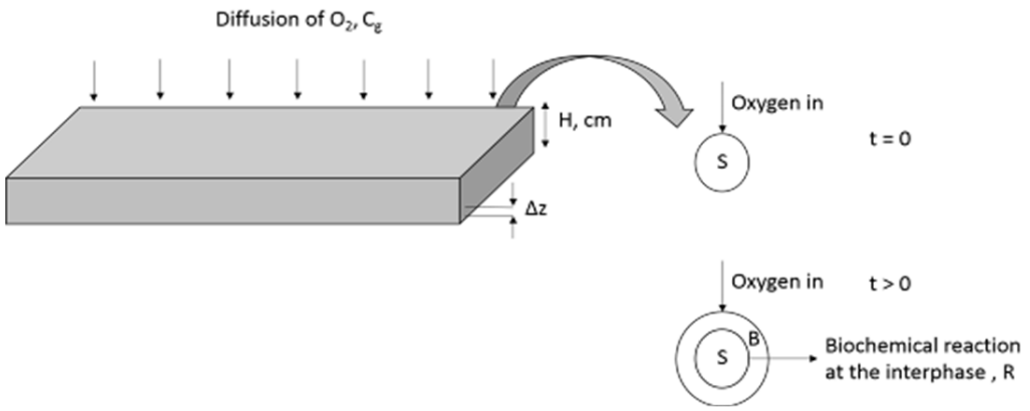

Fig. 1. Schematic representation of static tray solid state fermentor non-perforated at the bottom and diffusion of oxygen occurs only through the top. At t = 0, the thickness of the biomass is negligible and as fermentation progresses, t > 0, biomass film increase in thickness, which pose an additional resistance for transfer of oxygen

[S- Substrate particle, B – Expanding biomass film, R – Logistic equation, H – Total bed height, Δz – Small thickness of bed]

Validation of the developed model was carried out with the earlier data (Sugama and Okazaki, 1979) for the growth of *Aspergillus oryzae* cultured on solid media. The reaction rate R was calculated from the data using logistic equation. The other parameters are obtained from the literature and are given as follows: Maximum specific growth rate, μ_{max} = 0.3 h^{-1}, Effective diffusivity, D_e = 0.03 cm^2 s^{-1}, Yield coefficient, $Y_{X/S}$= 1.07 g g^{-1}.

The model developed for different kinetic rates are simulated and the oxygen concentration profiles were obtained for various parameters such as design parameter – substrate bed height (H), operational parameter - initial gas phase oxygen concentration (C_{O_2}) and kinetic parameter - saturation constant (K_s). Simulations were carried out for range of bed heights (2 to 9 cm). Oxygen concentration profile of pseudo steady state assumption by Finite difference method (in this work) was compared with profile solved by analytical solution (Rajagopalan and Modak [16]) for bed heights (2 to 9 cm), so we chose the same bed heights for easier comparison.

The porosity of the solid substrate bed is not uniform and that is reduced as fermentation proceeds. The change in porosity has an effect on oxygen concentration existing in the bed. Effect of porosity on oxygen concentration profiles was simulated for different kinetic rates.

3. RESULTS AND DISCUSSION

Earlier, Raghavarao and coworkers [16] developed a model to predict the oxygen profile in tray reactor with pseudo steady state assumption and considering zero order kinetics for consumption of oxygen. In reality, the assumption of steady state approximation is invalid because the concentration of oxygen varies with fermentation time [16]. In addition, oxygen is also one of the limiting nutrient in SSF and oxygen dependence may follow Monod's kinetics. Hence to have more insights to this phenomenon and better prediction of oxygen profiles in tray bioreactor, we developed a generalized model (Eqs. 2, 3 and 4). Oxygen concentration inside the substrate bed may vary with respect to design and operational parameters of the reactor and also with kinetic parameters of the organism. Thus simulation runs were carried out for different values of bed height (H) = 2, 5 and 9 cm (design parameter), oxygen concentration in the inlet air (C_{O_2}) 21%, 50% and 100% (operational parameter) and saturation constant (K_s) 1% and 8% of C_{O_2} (kinetic parameter). Porosity of the bed also plays a key role in determining the concentration of oxygen inside the bed. Variation of oxygen concentration for bed porosity 0.5 and 1 was also simulated for the experimental growth data of *Aspergillus oryzae* [18].

3.1 Prediction of Oxygen Concentration inside the Substrate Bed of SSF Tray Bioreactor with Pseudo Steady State Approximation

Raghavarao and coworkers [16] have developed a model to predict the oxygen profile with steady state assumption for zero order kinetics. In order to predict the oxygen profile for organisms following first and Monod kinetics, model equations has been developed (Eqs. 2 - 4) and represented in its dimensionless form in Eqs. 6 - 8. By assuming pseudo steady state, (i.e, the consumption of oxygen with respect to time is negligible when compared to the change in oxygen concentration with bed height, dC*/dt = 0), Eqs. 6 - 8 were reduced to second order ordinary differential equations Eqs. 11 - 13. Studies show that in tray bioreactor for SSF, the particle at different heights in the bed experiences different biofilm phase oxygen concentration due to diffusional limitations across the bed of solid particles [21]. To study the variation of oxygen with varying bed height, Eqs. 11 - 13 were simulated for different bed height (H = 2, 5 and 9 cm) and solved numerically using ode45. Variation of oxygen concentration inside the substrate bed at varying bed height for different reaction kinetics with pseudo steady state approximation is shown in Fig. 2. The oxygen concentration profile for zero order kinetics was depicted in Figs. 2a - 2c for different bed height H = 2, 5 and 9 cm respectively. The oxygen concentration profile generated based on current simulation (ode45) agreed with the profile as obtained via closed form solution [16]. The oxygen concentration profile for reactions with first order (Figs. 2d - 2f) and Monod kinetics (Figs. 2g - 2i) follows similar pattern as that of zero order reactions. Irrespective of the reaction kinetic equation, the oxygen concentration decreases as the height of the bed increases. This observation was in correlation with experimental findings, where higher biomass yield was obtained for small bed heights in which O_2 diffusional limitations are minimal [22].

Though dC*/dt = 0, reaction rate R, which is represented by logistic equation (Eqn. 1), is calculated as a function of time. This variation of oxygen concentration with respect to tray depth for different fermentation time helps in the prediction of oxygen limitation zone (tray depth at C*= 0) and time at which C* = 0 occurs. For zero order reaction, at bed height H = 9 cm, O_2 limitation (C* = 0) occurs at z* = 0.5 and at fermentation time t = 22 h (Fig. 2c). In case of first order reaction, at H = 9 cm, O_2 limitation occurs at an earlier fermentation time at t = 18 h and at z* = 0.6 (Fig. 2f). At fixed bed height, O_2 limitation occurs at early fermentation time for first order kinetics when compared to zero and Monod's kinetics. When reaction follows first order kinetics, rate depends on the concentration of oxygen, thus as fermentation proceeds, more oxygen is consumed and O_2 concentration inside the bed becomes low whereas in case of Monod kinetics, oxygen consumption depends not only on concentration of oxygen but also related to saturation constant K_s. Variation of oxygen concentration with K_s is discussed in section 3.4. Only at high substrate concentration, the reaction follows zero order kinetics, concentration of oxygen will remain high when compared to first order reaction kinetics.

For zero order kinetics (Bed height, H = 9 cm), at initial stage of fermentation (t= 10 and 12 h), concentration of oxygen is high and it does not vary much with tray depth (Fig. 2c). During exponential phase (t = 14 - 32 h), O_2 concentration inside the bed decreases with respect to tray depth and eventually becomes zero at particular depth. This can be attributed to the fact that low biomass consumes less oxygen and also the amount of biomass present initially is too low to cause diffusion limitation processes. Later, oxygen diffusion in the fungal mat becomes limiting as the thickness of the film increases [23]. At a later stage of fermentation (t = 34 h), the decrease in oxygen concentration reduces the growth of cells, that results in a decelerated growth phase [24]. Thus the bed experiences increase in O_2 concentration at later fermentation time. Bed height also affects the variation of O_2 concentration with tray depth. Oxygen concentration remains high along the tray depth for smaller bed height. Figs. 2g - 2i, shows very slight variation in O_2 concentration for reactions following Monod's kinetics with pseudo steady state assumption. It may be because of the negligence of change in concentration with respect to fermentation time. So we made an attempt to explore variation of O_2 concentration with unsteady state assumption (dC*/dt = 0).

3.2 Prediction of Oxygen Concentration inside the Substrate Bed of SSF Tray Bioreactor with Unsteady State Assumption

At exponential growth phase, oxygen decreases rapidly with increase in fermentation time. This change in O_2 concentration makes pseudo

steady state assumption invalid. The concentration of oxygen profile for varying dimensionless tray depth and dimensionless fermentation time is obtained by solving partial differential equations (Eqs. 6 - 8) by finite difference method. The comparative study of oxygen concentration profile with pseudo steady state approximation and unsteady state assumption will determine the suitability of the developed model.

In case of unsteady state assumption, Figs. 3a – 3i depicts the oxygen profile for varying tray depth and shows that the O_2 limitation occurs at an earlier fermentation time and at smaller tray depth when compared to the oxygen profiles with pseudo steady state approximation Figs. 2a – 2i. Reactions following zero order kinetics, at bed height H = 9 cm, O_2 limitation (C* = 0) occurs at z* = 0.3 and at fermentation time t = 22 h and also observed at an early fermentation time t = 12 h and at z* = 0.8 (Fig. 3c) but for pseudo steady state, O_2 limitation occurs at t = 22 h and at z* = 0.5 (Fig. 2c). For first order reaction, at H = 9 cm, O_2 limitation occurs at t = 22 h and at z* = 0.2 (Fig. 3f). The regions near the bottom of the substrate bed experiences lower concentration of oxygen, this is because the rate of consumption of oxygen in the film phase exceeds the rate of transfer of oxygen from the gas phase onto the biofilm and also due to diffusional limitation [21].

O_2 profiles for reactions following Monod's kinetics with unsteady state assumption (Figs. 3g – 3i) are compared with profiles of pseudo steady state assumption (Figs. 2g - 2i). It reveals that O_2 concentration decreases rapidly in case of unsteady state assumption for increasing bed height and tray depth. This profile is in agreement with the profile generated in earlier modeling work, where growth follows Malthus equation (dX/dt = μX) suggesting that variation of C* is influenced by fermentation time and tray depth [12]. In the current model, growth is assumed to follow logistic equation and C* profile

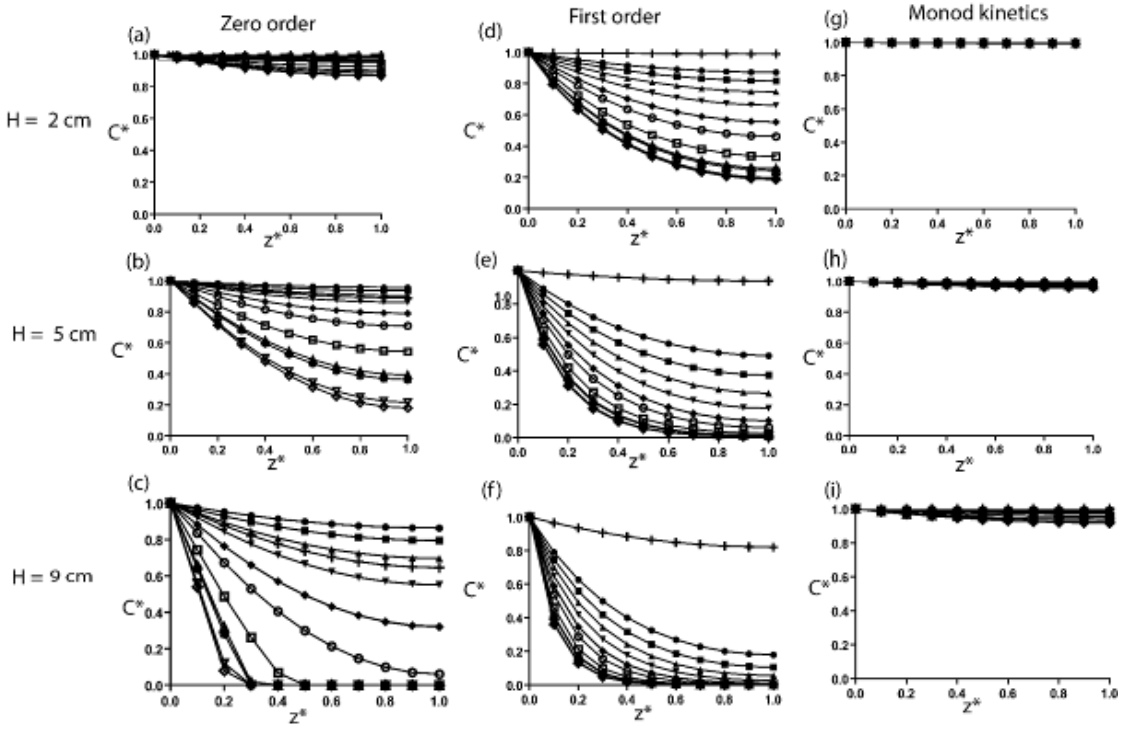

Fig. 2. Oxygen concentration profile for different reaction rate in solid state fermentation with pseudo steady state approximation. Simulated for different bed height, (H = 2, 5 and 9 cm) for zero order (a, b, c), first order (d, e, f) and Monod kinetics (g, h, i)

(● -10 h, ■ - 12 h, ▲ - 14 h, ▼ - 16 h, ◆ - 18 h, ○ - 20 h, □ - 22 h, △ - 24 h, ▽ - 26 h, ◊ - 28 h, ✳ - 30 h, ⋆ - 32 h, + - 34 h)

is generated for unsteady state assumption, which helps in prediction of C* at all stages of growth. Thus, change in O_2 concentration with time (dC*/dt*) also influence the prediction of oxygen concentration inside the bed as considered in the work of Rajagopalan and coworkers to predict the concentration of oxygen in the film phase [25].

3.3 Effect of Gas Phase Oxygen Concentration C_{O_2} on C* Inside the Substrate Bed

Oxygen limitation becomes more predominant when the initial concentration of oxygen is decreased. The existence of anaerobic conditions in the tray bed results in reduced biomass yield per mole of substrate consumed [26]. Increase in aerobic growth may be achieved via increasing the oxygen partial pressure in the gas phase, as higher interfacial oxygen concentration will increase the oxygen penetration depth [27]. Simulation runs were carried out by varying gas phase oxygen concentration C_{O_2}– 21%, 50% and 100% at fixed bed height (H = 2 cm, 5 cm and 9 cm) and for fermentation time t = 10 h and 28 h for reactions following zero order and Monod kinetics. Considering zero order reactions, when bed height is small (H = 2 cm), O_2 limitation does not occurs at low C_{O_2} (21% - air is supplied) even at the exponential growth phase, t = 28 h as shown in Fig. 4a. As bed height increases (H = 5cm), C* reaches zero and zone of zero of O_2 occur in the bed at lower tray depth only during exponential growth phase and not at early stage of fermentation (t = 10 h). Further increase in bed height (H = 9 cm) results in zero O_2 zone at early fermentation time and at higher tray depth if air is supplied into the bed. O_2 limitation at higher bed heights can be eliminated if O_2 enriched air or pure O_2 is supplied to the substrate bed as shown in Figs. 4b and 4c. As the initial oxygen concentration decreases, the duration of exponential phase reduces while that of deceleration phase increases [28].

Considering reaction following Monod kinetics, significant variation in C* with respect to C_{O_2} is clearly seen at higher bed heights. C* decreases gradually along the tray depth and becomes low at the bottom due to low O_2 transfer rate than consumption. Low C* at the bottom of the tray during early and exponential phases occurs only at higher bed height but it does not reach zero as shown in Figs. 4d – 4f. Variation of C* with

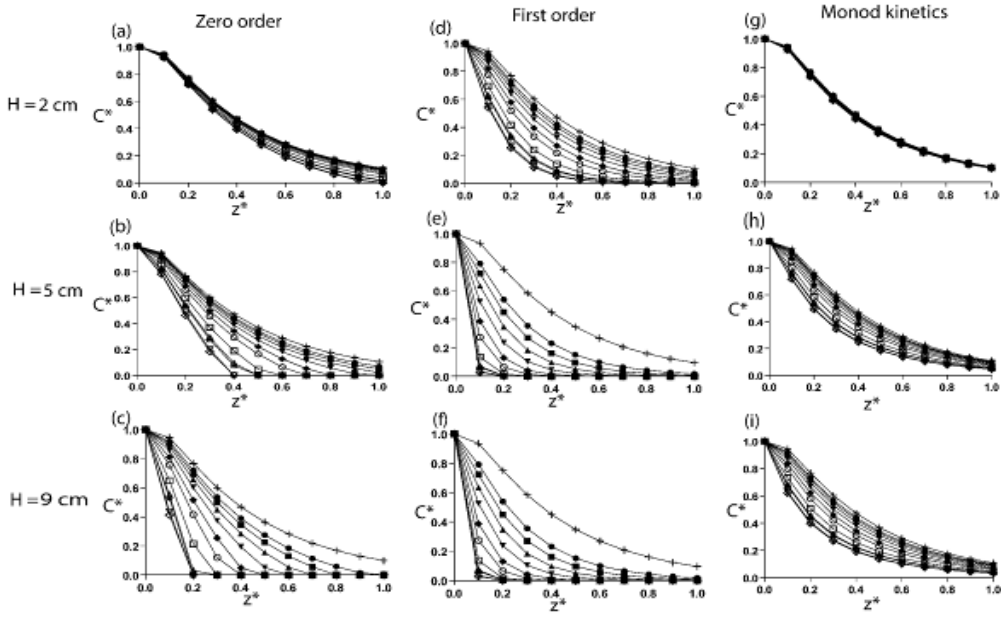

Fig. 3. Oxygen concentration profile for different reaction rate in solid state fermentation with time dependency. Simulated for different bed height, (H = 2, 5 and 9 cm) for zero order (a, b, c), first order (d, e, f) and Monod kinetics (g, h, i)

(● -10 h, ■ - 12 h, ▲ - 14 h, ▼ - 16 h, ◆ - 18 h, ○ - 20 h, □ - 22 h, △ - 24 h, ▽ - 26 h, ◇ - 28 h, ✳ - 30 h, ⋆ - 32 h, + - 34 h)

increasing CO2 is also influenced by kinetic parameter, saturation constant Ks (Eqn. 6). Eqn. 7 shows that the variation of oxygen inside the bed for first order reaction does not depend on gaseous phase oxygen concentration CO2. However, it depends on the oxygen saturation constant Ks.

It is also known that increasing oxygen concentration is quite costly and high oxygen concentration can lead to inhibition of microbial growth. Increase in aerobic growth can also occur via increasing the oxygen transfer rate from the gas phase to the microbial biomass which can be done by enlargement of the interface area between the gas phase and the moist biomass layer. Interfacial area can be increased by reducing the particle size or by pre-treatment of the substrate. It helps in making decision by providing leads for the choice and pre-treatment of substrate particles and composition of gaseous atmosphere [29]. Increased oxygen concentration in gaseous phase results in increased depth of the aerobic growth zone in the substrate bed and also within the liquid film. Therefore, in operating tray fermentors for SSF, more attention needs to be given to eliminate oxygen limitations since the oxygen concentrations will decrease from top to inside of the bed of solid particles as noted in the work of Rajagopalan and Modak [30].

3.4 Effect of Saturation Constant K_s on C* Inside the substrate Bed

Each microorganism exhibits different saturation constant K_s based on its affinity towards the substrate. Simulations were carried out to study the effect of saturation constant K_s (0.1%, 1% and 8% of C_{O_2}) on C* inside the bed for first and Monod's kinetics at fixed bed height (H = 2 cm, 5 cm and 9 cm) and are shown in Figs. 5a - 5c and Figs. 5d – 5f respectively. In case of first order reaction kinetics, C* in the substrate bed is high at early fermentation time and for increased K_s Zone of zero oxygen concentration varies with respect to saturation constant K_s and bed height. Figs. 5a - 5c depicts greater prevalence of C* at increased K_s and at lower bed height. Time and depth at which O_2 limitation occurs is also influenced by K_s for first order reactions. When K_s < 1%, depletion of oxygen occurs at lower tray depth and at early fermentation time, but for high value of K_s, C* is high, corroborating the fact that organism shows low affinity towards oxygen thus leaving excess oxygen inside the substrate bed.

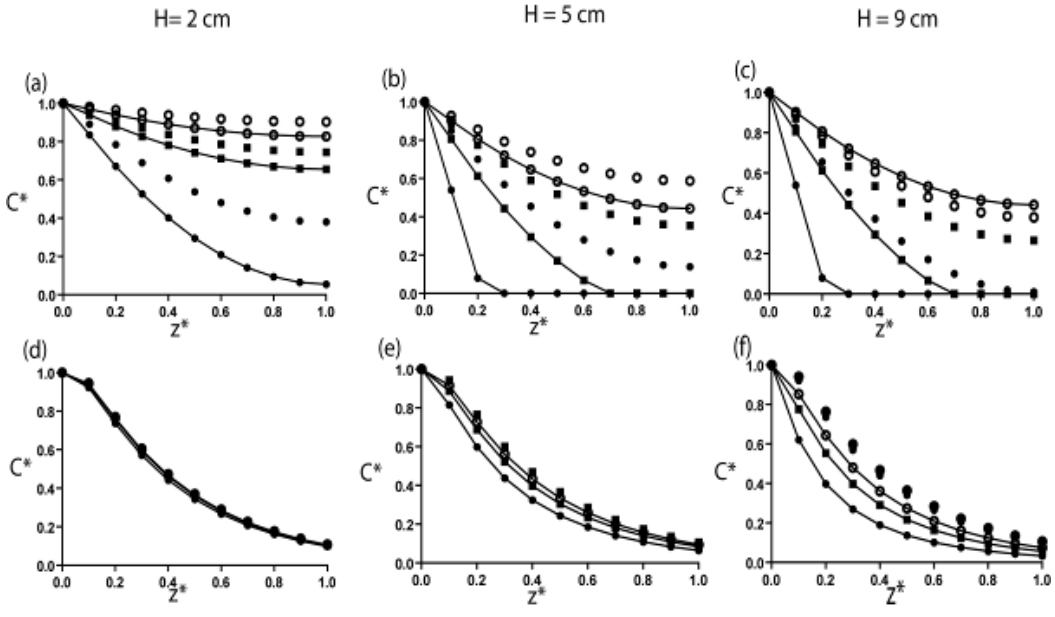

Fig. 4. Variation of oxygen concentration with tray depth for different initial oxygen concentration at varying bed height, H = 2 cm, 5 cm and 9 cm for zero order (a - c) and Monod kinetics (d – f)

(● - 21%, ■ - 50%, ○ - 100%) Symbols connected with solid lines shows the variation of C at t = 28 h, symbols without connecting lines shows variation at t = 10 h.*

Considering reactions following Monod kinetics, effect of K_s on C* is more explicitly seen only at higher bed height H = 5 cm and 9 cm as shown in Figs. 5e and 5f respectively. At lower bed height H = 2 cm, variation of C* along tray depth is shown in Fig. 5d and no significant variation in C* is observed for different K_s Diffusional limitation of O_2 can be the reason for variation in C* along the tray depth. C* inside the substrate bed for reaction rate following Monod kinetics varies as a function of bed height, fermentation time, tray depth, K_s and C_{O_2}.

3.5 Effect of Bed Porosity on C* Inside the Substrate Bed

In SSF, the porosity of the substrate bed is not constant and changes during the course of fermentation because of microbial growth on the substrate particle; which in turn affects the oxygen diffusion inside the bed [31]. In order to study this, simulations were carried out with various values of porosity. Figs. 6a – 6f, depicts the variation of O_2 concentration along the tray depth at bed porosity 0.5 and 1 for different reaction kinetics. O_2 concentration decreases with decreased porosity regardless of the reaction kinetics. Variation in oxygen concentration with bed porosity can be attributed to air or liquid filled voids. Pores filled with water undergo oxygen diffusional limitation but air filled pores prevents oxygen limitation [32]. Porosity of the bed decreases during static periods as biomass layer thickens and occupies the inter-particle spaces or because of high moisture content. Effect of higher moisture content was reported to involve decreased porosity, loss of particle structure, development of stickiness, reduction in gas volume, decreased gas exchange and lowered oxygen transfer due to decreased diffusion [33]. When porosity (ε = 1) is high, at bed height H = 5 cm, O_2 limitation occurs at t = 22 h and z* = 0.6 (Fig. 6a) for zero order reactions but when ε = 0.5, it occurs at t = 18 h and z* = 0.4 (Fig. 6b). Reactions following first order kinetics, when ε = 1, limitation occurs at t = 18 h and z* = 0.4 (Fig. 6c) and at decreased porosity (ε = 0.5) limitation of O_2 occurs at t = 16 h and z* = 0.3 (Fig. 6d). O_2 limitation occurs at early fermentation time for first order reactions. For reactions following Monod's kinetics, limitation occurs when bed porosity is low (Figs. 6e and 6f). Irrespective of the reaction kinetics, O_2 concentration decreases as the tray depth increases. The upper parts of the bed experiences high O_2 concentration and it decreases drastically as the depth increases. This may be due to the prevalence of increased porosity as a consequence of water and dry matter loss in the upper parts of the bed and accumulation of water near the plate drastically reduces the porosity [34] which in turn results in decreased oxygen concentration at the bottom of the tray.

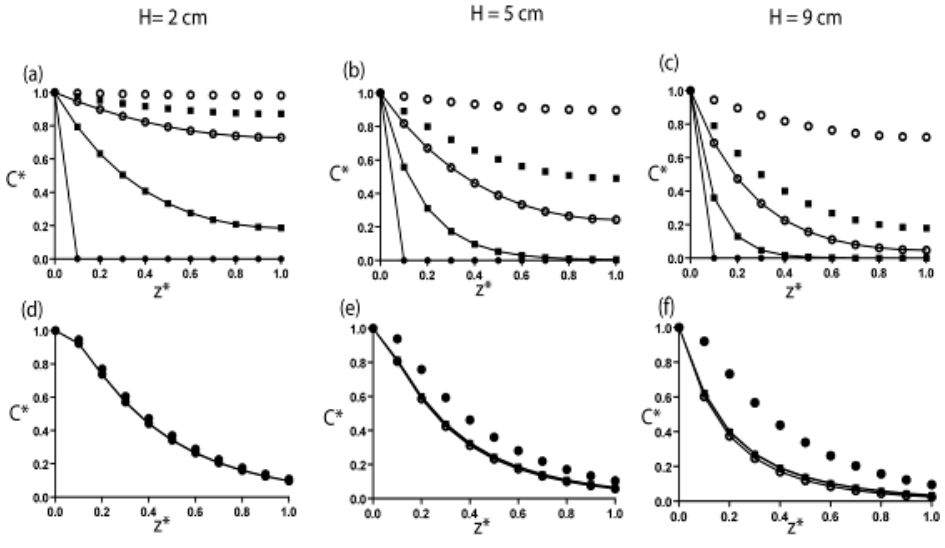

Fig. 5. Variation of oxygen concentration with tray depth for different saturation constant K_s at varying bed height, H = 2 cm, 5 cm and 9 cm for first order (a- c) and Monod kinetics (d –f).
(● - 0.01 %, ■ - 1%, ○ - 8%) Symbols connected with solid lines shows the variation of C at t = 28 h and symbols without connecting lines shows variation at t = 10 h*

3.6 Thiele Modulus

Thiele modulus measures the ratio of biochemical reaction rate to the rate of differential mass transfer within the solid, which can be useful to evaluate intraparticle mass transfer limitation. A dimensionless modulus equivalent to 'Thiele modulus' was derived for zero order Φ_0, first order Φ_1 and Monod kinetics Φ_M and was tabulated in Table 1. This dimensionless modulus helps in determination of control mechanism of the process. Variation in relative oxygen concentration C* with dimensionless tray depth was established for different values of Thiele modulus for zero, first and Monod kinetics (Figs. 7a - 7c). Oxygen concentration decreases as tray depth increases for all values of Φ. As Φ increases, the oxygen concentration decreases implying that the reaction rate is faster than the diffusion of oxygen. At lower Φ, the diffusion of oxygen occurs at a higher rate when compared to the reaction rate and that results in increased oxygen concentration in the substrate bed. Oxygen limitation occurs at higher values of Φ. C* variation in accordance to different values of Φ would have been useful in predicting the control mechanism and thus O_2 limitation inside the bed can be avoided.

Table 1. Thiele modulus for different reaction kinetics derived from mathematical model of oxygen concentration profile

Reaction kinetics	Thiele modulus
Zero order	$(H^2R/D_eC_{O_2}Y_{X/O_2})^{1/2}$
First order	$(H^2R/D_eK_sY_{X/O_2})^{1/2}$
Monod kinetics	$(H^2R/D_e C_{O_2}Y_{X/O_2})^{1/2}(1/1+\beta)[1+\beta \ln(\beta/1+\beta)]^{-1/2}$

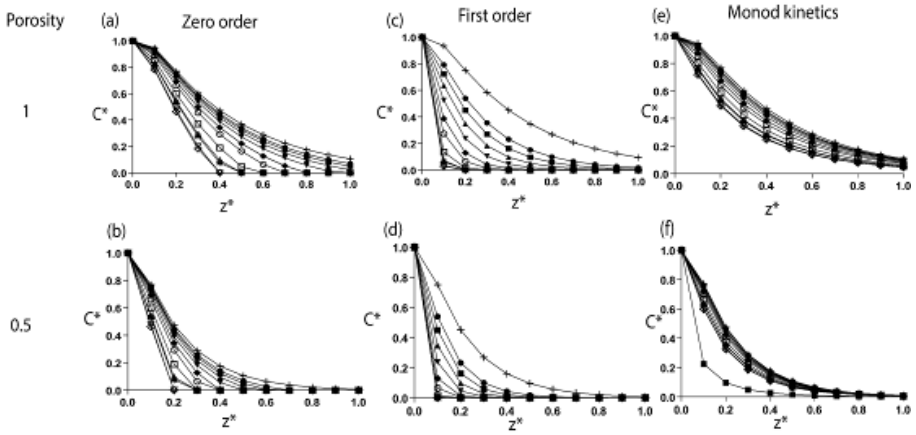

Fig. 6. Effect of porosity on oxygen concentration profiles for varying tray depth at bed height H = 5 cm for reaction following zero order (a, b), first order (c, d) and Monod kinetics (e, f).

(● -10 h, ■ - 12 h,▲- 14 h,▼- 16 h,◆ - 18 h, ○ - 20 h, □ - 22 h,△- 24 h,▽- 26 h,◊- 28 h, ✳ - 30 h, ★ - 32 h, + - 34 h)

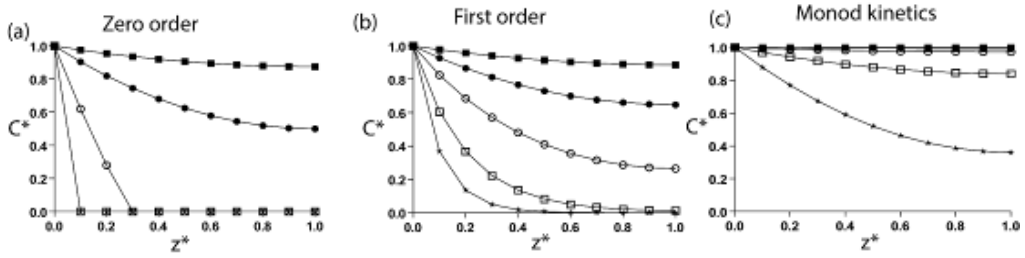

Fig. 7. Concentration profile of oxygen along the dimensionless tray depth for various values of Thiele modulus of zero order (a), first order (b) and Monod kinetics (c).

(■ - 0.5, ● - 1, ○ - 2, □ - 5, ★ - 10)

4. CONCLUSIONS

A mathematical model was developed considering various reaction kinetics (zero, first and Monod's) with unsteady state assumption to predict the oxygen profile inside the substrate bed of the static tray bioreactor SSF. C* predicted by this model was compared with the results of models with pseudo steady state assumption. It has been found that decrease in C* along the tray depth was noted in both models but at any fermentation time t, reduction in C* is high in reactions with unsteady state assumption. We can conclude that pseudo steady state approximation does not hold good for better prediction. Simulation results show that for higher bed height, C* inside the substrate bed is low, but for higher gas phase oxygen concentration and saturation constant, C* remains high. Simulation runs of this model aids in predicting the occurrence of O_2 limitation zone in tray bioreactor. Modeling and simulation studies of this work establishes relation between O_2 concentration and operational, design parameters of the reactor and kinetic parameters of the organism. We expect that this model will be useful in predicting the oxygen concentration prevailing inside the substrate bed and contributes in designing bioreactor for solid state fermentation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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