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Original Article

Substrate Diffusion Analysis in Immobilized Spherical Cell-Support Aggregate by Using of Least Square Method

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Abstract

In this study, the substrate diffusion in an immobilized spherical cell-support aggregate is studied and effects of various parameters are investigated on substrates profile. Analyses are performed by using of an analytical solution called the Least Square Method (LSM) and results are compared with numerical solution. The effects of effective diffusion coefficient (D_e), maximum specific growth rate (μm) and type of limiting substrate are studied on substrate concentration profile in immobilized *Nitrosomonas europaea* and *Nitrobacter agilis* microorganisms. Outcomes reveal that LSM is an appropriate method for analyze of biological non-linear equations. In the center of the spherical microbial support, the substrates concentration is minimums and with reducing μm or increasing *De*, substrate concentration profile gradient reduces.

Keywords: *Nitrosomonas europaea*, *Nitrobacter agilis*, LSM, Substrate Concentration

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Introduction

In recent years, using of immobilized cells is developed for various applications. Some of these applications are removing organic pollutants from contaminated ecosystems or producing of useful products such as ethanol [1-3] and many aerobic fungi have been immobilized for production of extracellular enzymes [4, 5]. Cell immobilization has several advantages including achieving to higher cell densities and increasing productivity respect to conventional suspension culture. Immobilization of mammalian cells within support can protect cells from destruction due to shear forces and simplify product separation and purification [6-8]. Despite of mentioned benefits for cells immobilization, there are disadvantages such as limitation of available space in the support for microorganisms growth [9]. Products and substrates diffuse from local environment within the biofilm layer and structure of biofilm is related to the motion of surface cells and existence of rate-limiting substrate [10, 11]. As regards to entrapment of cells in supports such as ca-alginatehas been performed for degradation of toxic substance since 1975, but diffusion limitations decreased productivity and environmental condition controlling [12-14] nitrate and nitrite diffusion into the spherical immobilized cell are studied for groundwater denitrification and quasi-steady-state model formulated for this reaction [15]. Immobilization of two morphologically different strains of anaerobic chytridin Ca-alginathas been studied for cellulolytic enzymes production and immobilization conditions have been optimized in order to fungal biomass increasing [16].

Experimental studies have showed that the biomass concentration in an immobilized cell support aggregate as non-uniform due to accumulation of biomass in the outer core of the support particle and this non-uniform distribution is affected on biological properties such as cell specific growth rate and effectiveness diffusion coefficient of the substrates and products [17-19]. The mathematical model have been derived based on uniform diffusivity in carrageen an gel beads and the effects of immobilized cells growing conditions upon biomass production are studied in support particles [20]. *E. coli* microorganism has been immobilized in agar membrane and substrate diffusion-reaction and cell reproduction has been modeled. The results showed that product inhibition was more effective on cell reproduction than substrate limitation [21].

Validation of a dynamic model has been studied for immobilized cells growth in kappa-carrageenan gel beads and simultaneous oxygen, nitrite, nitrate and ammonia conversion [22]. Macroscopic diffusion in immobilized cell supportcan be described by an effective diffusion coefficient (D_e) and this coefficient depends on the molecules diffusivity within the support phase (D_0) and the heterogeneous milieu of cells (D_c) [13, 23, 24]. There are several methods for evaluating molecular diffusivities in immobilized microorganism systems such as bead methods, diffusion chambers and holographic laser interferometry [25-27]. Many of nonlinear equations in the transport phenomena problems should be solved by analytical and numerical methods. Ozisik introduced some simple and accurate analytical techniques for solving nonlinear differential equations called the Weighted Residuals

Methods (WRMs). Collocation, Galerkin and Least Square Method (LSM) are examples of the WRMs [28, 29].
The main motivation of this paper is to solve mass balance one-dimensional differential equation by using of Least Square Method (LSM) and demonstrating of substrate concentration profile in diverse conditions. Effects of various parameters including effective diffusion coefficient, maximum specific growth rate and the type of limiting substrate are described on substrate profile in immobilized cells-support aggregate. With respect to using of analytical methods for biochemical differential equations dissolution remains unnoticed, the main advantage of this study is using of LSM which does not need any linearization or perturbation, and comparing of results with numerical solution.

Materials and Methods

Problem description and governing equation

Cell entrapment in a porous matrix protects them from the shear stress outside of the support particles. Physical entrapment of microorganisms inside a polymeric matrix (gel) is one of the most widely used methods for cell immobilization. The gels commonly used for cell entrapment are polymers such as K-carrageenan, agar, alginate [30, 31]. When cells are immobilized within suitable support, concentration of limitation substrate change in diverse layers. In this paper we assumed that *Nitrosomonas europaea* and *Nitrobacter agilis* cells are entrapped within spherical beads of k-carrageenan and microbial flocare formed. So, substrate should diffuse in cells aggregation to be used by inner layers cells. Parameters which include effective diffusion coefficient (D_e), maximum specific grow rate (μm) and limiting substrates type are effective on substrate concentration profile in immobilized *Nitrosomonas europaea* and *Nitrobacter agilis* cells and this study has tried to illustrate these parameters effects.
The following assumptions have been used for this model derivation:
1. Since the rate of biofilm thickness increasing is much slower than the rate of substrate consumption, the system conditions can be assumed to be at quasi-steady state for little periods of time. So the simplest case is to assume that the immobilized cell aggregation is at quasi-steady state and all of the cells inside the biofilm are in the same physiological state and average kinetic constant are used for the biotic phase.
2. Only immobilized cells convert substrate to product.
3. Support is considered as uniform sphere and cells are uniformly distributed within spherical support particle.
4. The external mass transfer limitation is negligible for the transport of the substrate.
On the basis of these assumptions, the governing equation of substrate diffusion rate within immobilized cell layer has been written based on following nonlinear differential mass balance [32]:

$$\frac{d^2\bar{S}}{d\bar{r}^2}+\frac{2}{\bar{r}}\frac{d\bar{S}}{d\bar{r}}=\frac{\phi^2\bar{S}}{1+\bar{S}/\beta} \tag{1}$$

$$\bar{S}=\frac{S}{S_0}\quad \bar{r}=\frac{r}{R_0}\quad \beta=\frac{S_0}{K_s}\quad \phi=R\sqrt{\frac{\mu_m X}{Y_s D_e K_s}} \tag{2}$$

Where $\bar{S}$ is the dimensionless substrate concentration, S_0 is the initial substrate concentration, $\bar{r}$ is the dimensionless radius, K_s is the saturation constant, μm is maximum specific growth rate, X is the biomass concentration, Y_s is the substrate yield and D_e is the effectiveness diffusion coefficient.

The appropriate boundary conditions are:

$$\bar{r}=1:\quad \bar{S}=1 \tag{3}$$

$$\bar{r}=0:\ \frac{d\bar{S}}{d\bar{r}}=0 \tag{4}$$

The cells and substrates properties of the immobilized cells are given in Table 1. Generally, these presented numerical values are used for clear explain of various parameters effect on substrate profile [22].

Analytical solution

There is an approximation method to solve ordinary differential equations called Least Square Method (LSM). Consider the following differential equation:

$$D\left(u\left(x\right)\right)=p\left(x\right) \tag{5}$$

Let consider the function $\tilde{u}$ an approximation of $\tilde{u}$, which is a linear combination of trial functions:

$$u\cong\tilde{u}=\sum_{i=1}^{n}c_i\varphi_i \tag{6}$$

By substituting into the differential equation, an error or residual will exist:

$$R\left(x\right)=D\left(\tilde{u}\left(x\right)\right)-p\left(x\right)=0 \tag{7}$$

The notion in LSM is to force the residual to zero, so:

$$\int_X R\left(x\right)W_i\left(X\right)dx=0,\qquad i=1,2,\ldots,n \tag{8}$$

Where $w(x)$ is weight function and n is the number of unknown constants c_i in $\tilde{u}$. The result is a system of n algebraic equations for obtaining the unknown constants c_i. If the continuous summation of all the squared residuals is minimized, the rationale behind the LSM's name can be seen:

$$S=\int_X R\left(x\right)R\left(x\right)dx=\int_X R^2\left(x\right)dx \tag{9}$$

For obtaining the minimum of the function S, the derivatives of S with respect to all constants must be zero.

$$\frac{\partial S}{\partial c_i} = 2\int_X R(x)\frac{\partial R}{\partial c_i}dx = 0 \quad , \qquad i = 1,2,\ldots,n \tag{10}$$

By comparing Eqs (6) and (10), the weight functions are

$$W_i = 2\frac{\partial R}{\partial c_i} \tag{11}$$

Some advantages of LSM comparing to other methods are presented in [33, 34]. Here, we apply the LSM on the present problem. We should first choose a trial function. Because trial function must satisfy the boundary conditions (Eq. (3) and (4)), so it will be assumed as,

$$S(r) = 1 + c_1(1-r^2) + c_2(1-r^3) + c_3(1-r^4) + c_5(1-r^6) \tag{12}$$

By combining the above equation and Eq. (1), residual function will be found and via substituting the residual function into Eq. (10), a system of equation with five equations will appear and by solving this set of equations, coefficients $c_1,\ldots,c_5$ will be obtained. The analytical solution of the problem is in the following form for $\phi = 1, \beta = 1$:

$$S(r) = 0.9191 + 0.0798r^2 + 0.0r^3 + 0.0011r^4 + 0.000001r^5 - 0.0001r^6 \tag{13}$$

To validate our solution and find the accuracy of the method, we compared the results of the LSM and numerical solution in the Table 2. The numerical solution is performed by using the algebra package Maple 15.0, to solve the present case. The package uses a fourth–fifth order Runge-Kutta–Fehlberg procedure for solving nonlinear boundary value problem (*BVP*). The algorithm is proved to be precise and accurate in solving a wide range of mathematical and engineering problems. As we can see in Table 2, the results of LSM have an excellent accuracy and order of the error is about 10^{-6} to 10^{-5}.

Result and Discussion

In this study Least Square Method is applied to obtain an explicit analytical solution of the substrate diffusion equation in an immobilized cell-support aggregate. In the following analysis, all parameters are kept constant except for one and the effect of this parameter is studied on the substrate concentration profile.

The effective diffusion coefficient (D_e) is affected by various parameters such as cell concentration (X) [23] for this reason constant values of cell concentrations are applied for demonstration of substrates concentration profiles in Figures 1-10.

Effects of the effective diffusion coefficients (D_e^{NO2}) on concentration profile of NO_2 as limiting substrate are showed in Figure 1 for *Nitrobacter agilis* when X=1 kg/m3.Substrate concentration reduces with approaching to the center of spherical bacterial aggregation. Increasing of effective diffusion coefficient reduces substrate profile gradient since substrate can diffuse within cells easily (Fig. 1).

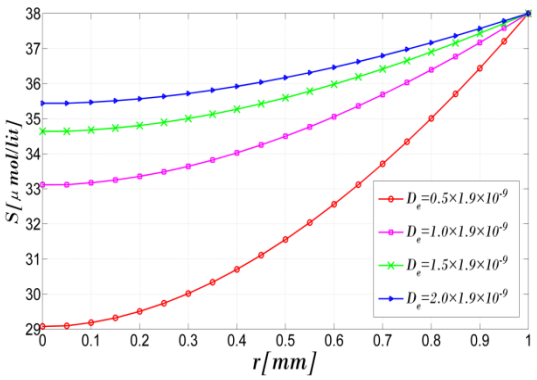

Figure 1. Effect of the D_e^{NO2} on NO_2 concentration profile for immobilized *Nitrobacter agilis*.

Considering the fact that saturation constant (K_s) is related to the type of substrate and microorganism, O_2 profile is demonstrated in Figure 2 for *Nitrobacter agilis* when X=1 kg/m3. With respect to $D_e^{O_2} \rangle D_e^{NO_2}$ in similar conditions, but O_2 profile gradient is more than NO_2since $K_S^{NO2,Nb} > K_S^{O2,Nb}$ (Table 1).

Table 1. The numerical value of model parameters.

Nitrobacter agilis (Nb)	$\mu_{max}^{Nb} = 1\times10^{-5}\,s^{-1}$	$K_s^{NO2,Nb} = 0.36\,molm^{-3}$ $K_s^{O2,Nb} = 17\times10^{-3}\,molm^{-3}$	$Y_{x/s}^{Nb} = 0.04$
Nitrosomonas europaea (Ns)	$\mu_{max}^{Ns} = 1.59\times10^{-5}\,s^{-1}$	$K_s^{NH4,Ns} = 1.25\,molm^{-3}$ $K_s^{O2,Ns} = 5.05\times10^{-3}\,molm^{-3}$	$Y_{x/s}^{Ns} = 0.12$
$D_e^{O2} = 2.83\times10^{-9}m^2s^{-1}$	$D_e^{NH4} = 2.2\times10^{-9}m^2s^{-1}$	$D_e^{NO2} = 1.9\times10^{-9}m^2s^{-1}$	$R = 1.0\,mm$
$X_0 = 1Kgm^{-3}$	$X_{mean} = 6.5Kgm^{-3}$	$S0 = 38.0\ \mu mol\,lit^{-1}$	

Therefore, as regards to D_e and K_s have inverse effect on substrate profile gradient, but K_s has more effect than D_e in this conditions. Increasing diffusion coefficient of O_2 reduces difference of substrate concentration between the bulk medium and the center of immobilized cells support.

Effects of $D_e^{NH_4}$ as nitrogen source nutrient on NH_4 concentration profile has been studied in Figure 3 for *Nitrosomonas europaea* when X=1 kg/m^3.

It's obvious that increasing of $D_e^{NH_4}$ makes substrate diffusion increases within immobilized cell support particle aggregate. O_2 concentration profile is demonstrated in Figure 4 for *Nitrosomonas europaea* (X=1 kg/m3) and responds are similar to Figure 2.

Table 2. Comparison of the LSM results and numerical solution for $\phi = 1, \beta = 1$.

	LSM (Eq. (13))	Numerical Solution	Error
r=0	0.919109	0.919095	1.42E-5
r=0.1	0.919908	0.919914	6.20E-6
r=0.2	0.922304	0.922294	1.00E-5
r=0.3	0.926302	0.926289	1.29E-5
r=0.4	0.931908	0.931897	1.13E-5
r=0.5	0.939132	0.939122	9.95E-6
r=0.6	0.947985	0.947975	9.62E-6
r=0.7	0.958480	0.958473	6.90E-6
r=0.8	0.970635	0.970627	7.48E-6
r=0.9	0.984468	0.984456	1.18E-5
r=1	1.0	1.0	0.0

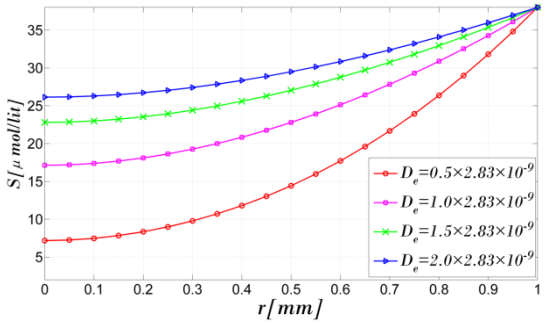

Figure 2. Effect of the D_e^{O2} on O_2 concentration profile for immobilized *Nitrobacter agilis*.

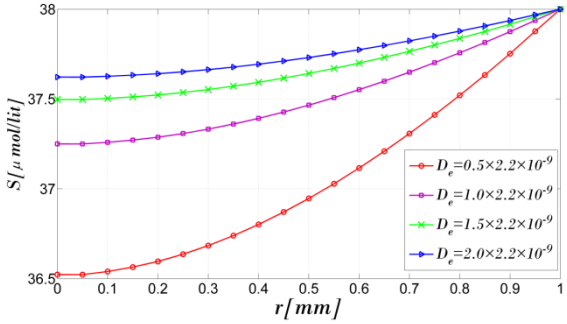

Figure 3. Effect of the D_e^{NH4} on NH_4 concentration profile for immobilized *Nitrosomonas europaea*.

Cell specific growth rate constant is a way of measuring how fast the cells are dividing in a culture. In Figure 5a and b, effects of μm have been show on substrate concentration profile for *Nitrobacter agilis*. It's obvious that with increasing maximum specific growth rate, substrate consumption increases and thus gradient of limiting substrate concentration profile (NO_2 and O_2) increases (X=1 kg/m^3). Effects of specific growth rate have been studied on substrate diffusion for *Nitrosomonas europaea* in Figure 6a and b for X=1 kg/m^3. This figure confirms our appointment about effects of maximum specific growth rate on substrate concentration profile.

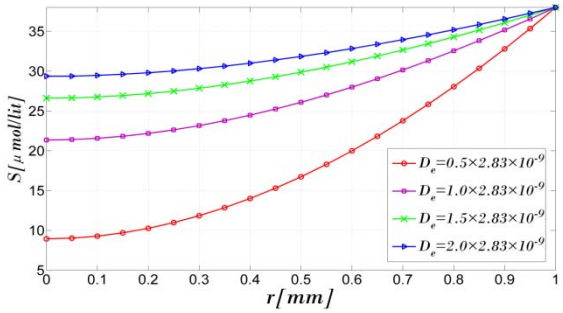

Figure 4. Effect of the D_e^{O2} on O_2 concentration profile for immobilized *Nitrosomonas europaea*.

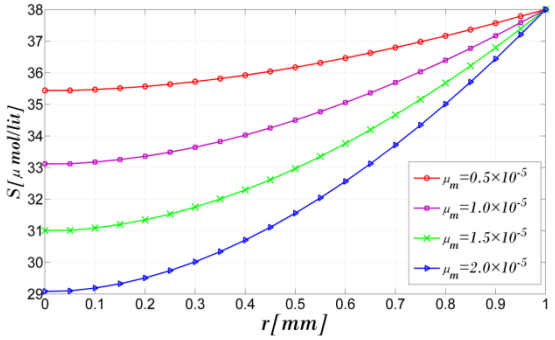

Figure 5a. Effect of *Nitrobacter agilis* specific growth rate (μm) on NO_2 concentration profile.

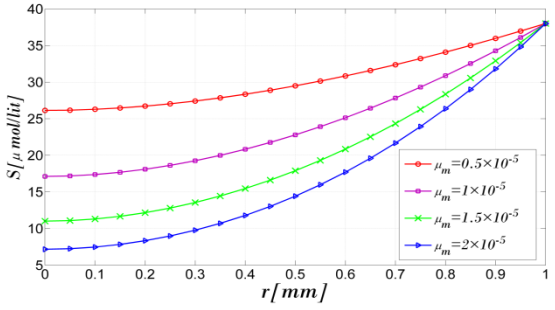

Figure 5b. Effect of *Nitrobacter agilis* specific growth rate (μm) on O_2 concentration profile.

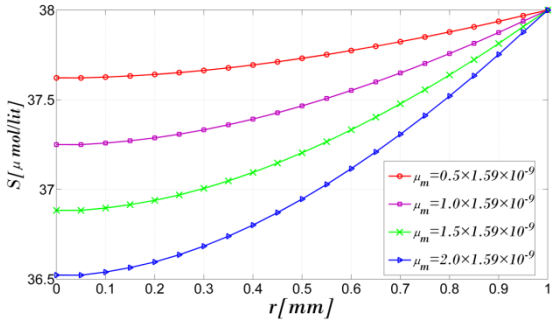

Figure 6a. Effect of *Nitrosomonas europaea* specific growth rate (μm) on NH_4 concentration profile

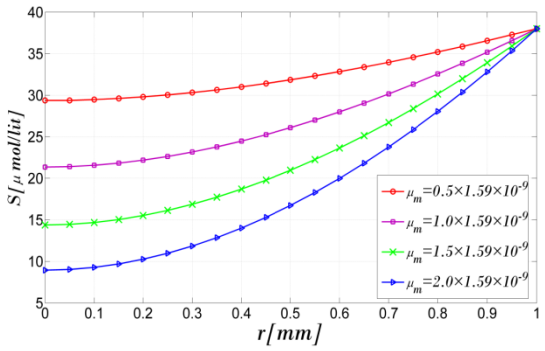

Figure 6b. Effect of *Nitrosomonas europaea* specific growth rate (μm) on O_2 concentration profile.

Comparison of O_2 concentration profile in *Nitrobacter agilis* and *Nitrosomonas europaea* are demonstrated in Figure 7 (X=1 kg/m^3). It's obvious that O_2 diffusion within immobilized *Nitrosomonas europaea* is more than *Nitrobacter agilis* due to diverse properties of these two microorganisms (Table 1). In Figure 8 diffusion of nitrogen source nutrient (NO_2, NH_4) are compared for *Nitrobacter agilis* and *Nitrosomonas europaea*.

NH_4 diffusion within *Nitrosomonas europaea* is more than NO_2 diffusion in *Nitrobacter agilis* when X=1 kg/m^3. This result shows that with using of NH_4 as nitrogen source nutrient, required nitrogen of cells is provided in inner layers of spherical cell-support aggregate.

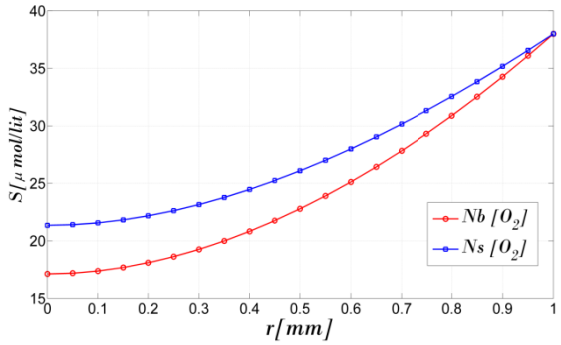

Figure7. Comparison of O_2 concentration profile for *Nitrobacter agilis* and *Nitrosomonas europaea*.

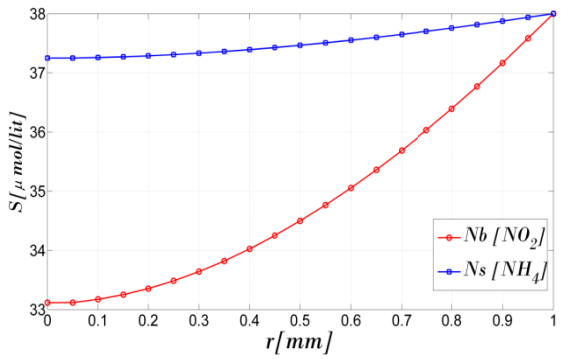

Figure 8. Comparison of nitrogen source concentration profile for *Nitrobacter agilis* and *Nitrosomonas europaea*.

Comparison of oxygen and nitrogen source substrate has been performed in figure 9. O_2 and NO_2 concentration profile are showed for *Nitrobacter agilis* in spherical microbial aggregation when X=6.5 kg/m^3. Substrates concentration are minimums in r=0 and O_2 profile gradient is more than NO_2.This figure state that oxygen diffusion is less than nitrogen source nutrient and in center of support may cell metabolism shift to anaerobic metabolism.

As seen in Figure10, O_2 and NH_4 concentration are minimums in the center of immobilized *Nitrosomonas europaea* when X=6.5 kg/m^3. NH_4 diffusion within spherical cell aggregation is more than O_2 and so, its profile gradient is less than oxygen.

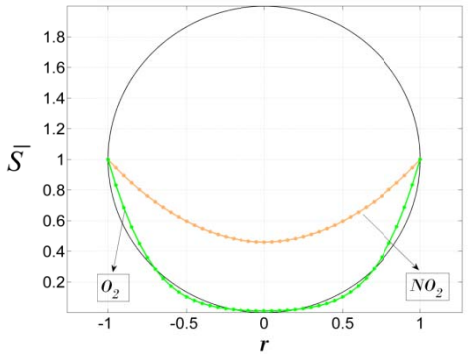

Figure 9. Comparison of O_2 and NO_2 concentration profile for Nitrobacteragilis.

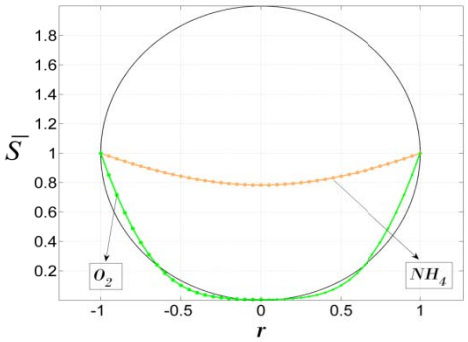

Figure 10. Comparison of O_2 and NH_4 concentration profile for *Nitrosomonas europaea*

Conclusion

Due to mass balance for immobilized cells within spherical cell-support aggregate is non-linear differential equation, a significant challenge is obtaining of the analytical solution for this equation. In this work the effects of various parameters such as effective diffusivity coefficient and maximum specific growth rate have been studied on diverse substrate profile for *Nitrobacter agilis* and *Nitrosomonas europaea* microorganisms by using of Least Square Method.

Results showed that the LSM and numerical solution had excellent agreement. In spherical microbial floc with approaching to the center of sphere, substrate concentration reduces and its gradient is function of media and microorganism properties. By effective diffusion coefficient increasing, substrate profile gradient reduces

but reducing of microorganism specific growth rate increases substrate profile slope within spherical microbial floc.

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