# Modeling of Microbial Interactions using Software and Simulation of Stable Operating Conditions in a Chemostat

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## ABSTRACT
The use of computer software in the fields of engineering, technology and management has become inevitable these days. Engineering problems are tedious and time consuming to solve manually due to higher complexity. In this perspective mathematical bioscience is an emerging field that involves formulation of biological concepts in terms of equations and application of computers to solve them. Though the biological systems are very complex and beautifully constructed they obey the rules of chemistry and physics that make them susceptible to engineering analysis. This forms the basis for bioprocess modeling optimization and simulation which can be accomplished using software. In the microbial world, varieties of species are available and both in natural systems and commercial applications mixed culture operations play a vital role. In such case interaction among them decides the output of the system and five patterns of interactions (namely neutralism, amensalism, competition, commensalism, mutualism, predation and parasitism) are observed so far. In the present work an innovative and unified approach is developed to characterize these patterns of interactions among microorganisms for two species interaction. The models for pure and mixed culture growth were derived from experimental data in batch mode using CFTOOL kit in MATLAB 7.1. The differential equations were solved using ODE SOLVER in MATLAB 7.1 and the simulation studies for continuous operations were carried out using C++ software. The simulated results and their interpretations are obtained using surface plots drawn using MINITAB software.

## Keywords
Interaction, Chemostat, Dilution rate, cftool, ode solver, surface plot, MATLAB, MINITAB

## 1. INTRODUCTION
Mathematical Bioscience is the application of mathematical modelling and mathematical techniques to get insight into the problems of biosciences. Study of genetic characteristics, analysis of optimal utilization of renewable resources, kinetic studies in biochemical systems that include enzymatic activities, etc are few instances of this area. The mathematical formulations in biosciences include ordinary and partial differential equations, difference equations, differential – difference equations, integral equations, integro - differential equations, delay - differential equations, matrix theory, graph theory, Combinatorial theory and calculus of variations. These systems of equations can be solved only with the help of computers.

Biochemical reactions involve microorganisms either as single species or multi species. The natural cycles of carbon, nitrogen, oxygen and numerous other elements on our planet all require the active participation of many different microorganisms [1]. Among commercial processes, biological wastewater treatment, cheese manufacture, production of ethanol, hydrogen, etc. are few examples where multiple microbial species are required. Interactions between microbial populations can be recognized as negative interactions in which one or both of the species in inhibited due to association and positive interactions where the association is beneficial for either of the population or both [3,5,6,7]. The various interaction patterns are neutralism, amensalism, competition, commensalism, mutualism, predation and parasitism. The behaviour and performance of the mixed species system greatly depends on the type of interaction among them [9,10,11]. The present investigation aims to develop a new methodology to describe the mixed microbial population kinetics incorporating these interaction phenomena in terms of mathematical equations and to solve them using computer. This is done by obtaining the growth equations for interacting microorganisms in batch scale and extending them to continuous process using chemostat model. The objective is to determine the stable operating conditions of a chemostat without washout of any of the species.

## 2. MATHEMATICAL MODELS

The analysis of multiple interactive microbial populations is a fascinating area of research that involves mathematical modelling to quantify the several interactive effects in describing the growth rates of the interacting species in mixed culture. Mathematical model in bioprocesses is defined as an equation that relates the influence of various parameters to cell growth (x) and consumption of the nutrients (s) by the microorganisms. Equations that are used to describe cell growth are logistic model, Monod model, Andrew's model whereas for nutrient depletion substrate utilization kinetics is commonly used. In the present study the logistic model is used for cell growth and the interaction effects are given as specific functions depending on the interaction mechanism.

### 2.1 The Logistic Model
The growth kinetics of microorganisms in the logarithmic phase which attains stationary phase is well explained by the classical logistic model [4] proposed by Verlhurst, Pearl and Reed which included an inhibiting factor to the population growth with the assumption that inhibition is proportional to the square of the microbial concentration $(x_i)$. This is given by

$$\frac{dx_i}{dt} = k_i x_i (1 - \beta_i x_i) \qquad (1)$$

This is a Ricatti equation which can be easily integrated to give the logistic curve. The constant terms $k_i$ and $\beta_i$ that appear in equation (1) represent the logistic constants. The logistic curve is sigmoidal and leads a stationary population of size $x_{is} = 1/\beta_i$. Hence it can be inferred that, higher the value of $\beta_i$, lower is the stationary phase concentration obtained.

Mathematical modelling from experimental data and simulation studies based on the models find an important tool in the design of any process for its implementation and control. In bioprocesses involving diverse microbial populations, the performance and behaviour of the systems depends not only on the growth characteristics of the individual species but also on the interaction patterns among them. Appropriate design of mixed microbial systems in both batch and continuous modes of operation and process schemes can be developed only through better understanding of the interaction phenomena and development of suitable models.

During mixed culture operations the growth of all species depends not only on the environmental conditions and the available nutrients for each species but also on the interaction among them. Since our study is limited to two species interaction, the general equations for describing the growth rates in associated form for the two species ($x_1$ and $x_2$) and the balances for substrates ($s_1$ and $s_2$) in batch case are given by equations (2) and (3). These equations apply when $s_1$ forms the limiting nutrient for species 1 and $s_2$ forms the limiting nutrient for species 2. And there is no significant effect by $s_2$ on $x_1$ and $s_1$ on $x_2$.

$$\frac{dx_1}{dt} = a_{11} f_{11}(x_1, s_1) + a_{12} f_{12}(x_1, x_2)$$

$$(2) \quad \frac{dx_2}{dt} = a_{22} f_{22}(x_2, s_2) + a_{21} f_{21}(x_1, x_2) \qquad (3)$$

where $f_{11}$ and $f_{22}$ represent the pure culture growth patterns of species 1 and 2 respectively and $f_{12}$ and $f_{21}$ give the interactive effect of species 2 on 1 and 1 on 2 respectively. All possible combinations of interactions namely neutralism, amensalism, competition, commensalism, mutualism, predation and parasitism defined in this manner are shown in Table 1[4].

In an amensal relationship, the growth of one species is inhibited by the presence of another [5, 12]. Competition represents a negative relationship between two populations in which both populations are adversely affected with respect to their survival and growth [3, 6]. In a commensal relationship, one population benefits while the other remains unaffected [8]. In mutualistic interaction, growth of both species is enhanced by the presence of each other [2]. In predation one species forms the food for the other [7].

**Table 1. Classification of pairwise interactions based on the sign of the entries $a_{12}$ and $a_{21}$**

| | | Effect of species 2 on species 1 (sign of $a_{12}$) | | |
|---|---|---|---|---|
| | | - | 0 | + |
| **Effect of species 1 on species 2 (sign of $a_{21}$)** | - | -- competition | -0 amensalism | -+ predation |
| | 0 | 0- amensalism | 00 neutralism | 0+ commensalism |
| | + | +- predation | +0 commensalism | ++ mutualism |

## 2.2 Chemostat

A chemostat [4] is a well-stirred vessel used for getting a steady supply of microorganisms whose effective volume is denoted as V. The chemostat is fed with the medium that contains an excess of all nutrients required by the microorganisms except one nutrient which is called the growth-limiting factor since its shortage reduces the growth rate of microorganisms.
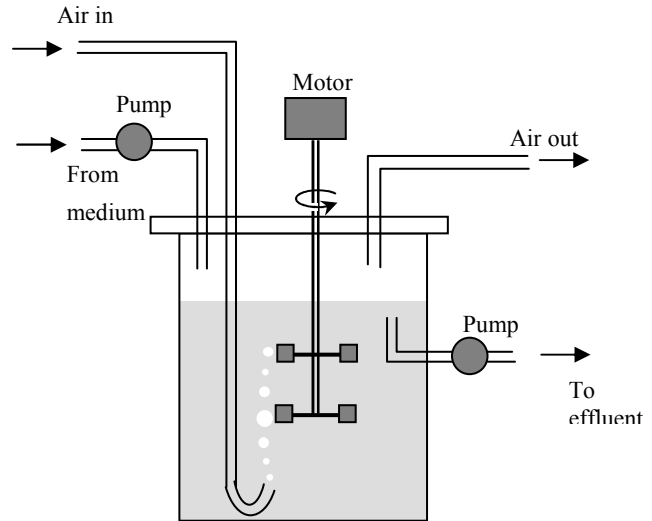


**Fig.1 Schematic representation of chemostat**

An important feature of chemostat cultivation is the dilution rate (D), defined as the volume (F) of nutrient medium supplied per unit time divided by the volume (V) of the culture. During chemostat cultivation, equilibrium is established (steady state) at which the growth rate of the cells equals the dilution rate. The growth rate of the microorganisms is purely based on the dilution rate. The concentration of the cells (biomass) in the chemostat is dependent on the concentration of the growth-limiting nutrient in the medium feed.

## 3 MODELING AND DISCUSSIONS

### 3.1 Amensalism

System: *Pseudomonas aeruginosa* (1) and *Micrococcus luteus* (2)
The experimental studies on the pure and mixed culture operations of the bacterial system *Pseudomonas aeruginosa* and *Micrococcus luteus* proved the existence of amensal interaction against luteus species. The experiments were carried out with various initial substrate concentrations (0, 100, 200, 300 and 400 mg/ml) to study its influence on the pure and mixed culture growth. Suitable models were developed to predict the pure and associated growth rates based on equations (2) and (3) and extended to continuous culture. These are given as

$$\frac{dx_1}{dt} = -Dx_1 + k_1 x_1 (1 - \beta_1 x_1) \qquad (4)$$

$$\frac{dx_2}{dt} = -Dx_2 + k_2 x_2 (1 - \beta_2 x_2) - a_{21} x_1 x_2 \qquad (5)$$

The interaction effect of species 2 on 1 is negligible and that for the second species, the interaction function $f_{21}(x_1, x_2)$ is found to be second order. Under batch case $D = 0$ and for pure culture $a_{21} = 0$. The logistic constants obtained in all the cases for the two species are reported in Table 2.

**Table 2. Logistic constants for *Pseudomonas aeruginosa* and *Micrococcus luteus***

| Initial glucose concentration (mg/ml) | $k_1$ (h$^{-1}$) | $\beta_1$ (millions cfu/ml)$^{-1}$ | $k_2$ (h$^{-1}$) | $\beta_2$ (millions cfu/ml)$^{-1}$ |
|---|---|---|---|---|
| 0 | 0.2264 | $3.59 \times 10^{-3}$ | 0.1751 | $5.34 \times 10^{-3}$ |
| 100 | 0.2338 | $3.23 \times 10^{-3}$ | 0.2098 | $4.64 \times 10^{-3}$ |
| 200 | 0.2443 | $2.70 \times 10^{-3}$ | 0.2104 | $3.43 \times 10^{-3}$ |
| 300 | 0.2734 | $2.62 \times 10^{-3}$ | 0.2413 | $3.02 \times 10^{-3}$ |
| 400 | 0.2305 | $2.75 \times 10^{-3}$ | 0.1960 | $4.94 \times 10^{-3}$ |

The k values remain almost same for both the species and hence the average values are taken (pseudomonas: 0.2417 h$^{-1}$ microccus: 0.2066 h$^{-1}$). However β values decrease with increasing glucose concentrations for both the species and this trend can be expressed by the cubic polynomials. $\beta_1$ and $\beta_2$ in terms of $s_0$, the initial glucose concentration are given by

$$\beta_1 = 0.03167s_0^3 - 0.008786s_0^2 - 0.003652s_0 + 0.003602 \quad (6)$$

$$\beta_2 = 0.2367s_0^3 - 0.09886s_0^2 + 0.0006762s_0 + 0.005337 \quad (7)$$

The interaction parameter too varies with respect to the initial substrate concentration as a cubic polynomial.

$$a_{21} = -0.009367s_0^3 + 0.003922s_0^2 + 0.00032s_0 + 0.000297 \quad (8)$$

The polynomial equations (6) to (8) are obtained by using cftool kit available in the MATLAB 7.1 software. The system of equations (4) to (8) are solved using ODE solver in MATLAB 7.1.Runge Kutta's numerical integration execution is available in this software as 'ode23' and 'ode45'. It was observed that the model was able to fit the experimental data with greater accuracy which is evident from the regression coefficient ($R^2 > 0.94$). The comparison between experimental and predicted values of the growth of the two species from the above equations is presented in figures 2 and 3 respectively.
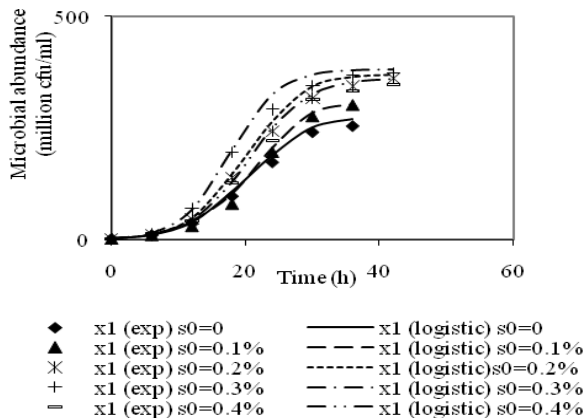


**Fig. 2 Growth curve of *Pseudomonas aureginosa* in pure culture with varying substrate concentrations**
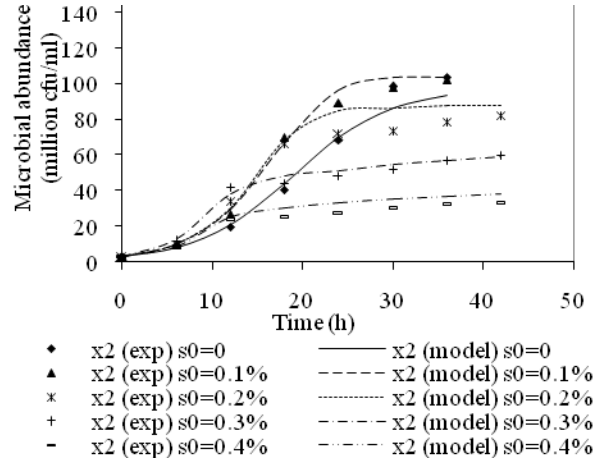


**Fig.3 Growth curve of *Micrococcus Luteus* in mixed culture with varying substrate concentrations**

The chemostat model equations are used to simulate the range of dilution rates to be maintained for coexistence of both the species for the various initial concentrations and the respective steady state microbial concentrations that would be obtained (Table 3). The simulation was carried out in C++ language.

$$x_{1s} = \left(1 - \frac{D}{k_1}\right)\frac{1}{\beta_1} \quad (9)$$

$$x_{2s} = \left(1 - \frac{(D + a_{21}x_{1s})}{k_2}\right)\frac{1}{\beta_2} \quad (10)$$

**Table 3. Range of the operating conditions for coexistence of both the species in a chemostat**

| Initial glucose concentration (mg/ml) | Maximum limit for D (h$^{-1}$) | $x_{1s}$ (million cfu/ml) Min | $x_{1s}$ (million cfu/ml) Max | $x_{2s}$ (million cfu/ml) Min | $x_{2s}$ (million cfu/ml) Max |
|---|---|---|---|---|---|
| 0 | 0.146 | 98.92 | 278.5 | 0.35 | 100.63 |
| 100 | 0.185 | 64.62 | 309.59 | 0.005 | 93.49 |
| 200 | 0.156 | 133.87 | 370.37 | 0.106 | 83.28 |
| 300 | 0.156 | 164.51 | 381.68 | 0.043 | 59.82 |
| 400 | 0.113 | 183.37 | 363.63 | 0.145 | 34.55 |

## 3.2 Competition

System: *Escherichia coli* (1) and *Staphylococcus aureus* (2)
The competitive interaction of the systems E.coli and S. aureus 255 and E.coli and S. aureus 261 based on the literature data [8] for three different temperatures viz. 15, 30 and 44 ºC were modelled and given by equations (11) and (12). Under pure culture the second terms in each of these equations become zero. For batch case D = 0.

$$\frac{dx_1}{dt} = -Dx_1 + k_1 x_1 (1 - \beta_1 x_1) - a_{12} x_2{}^2 \qquad (11)$$

$$\frac{dx_2}{dt} = -Dx_2 + k_2 x_2 (1 - \beta_2 x_2) - a_{21} x_1{}^2 x_2 \qquad (12)$$

The values of the logistic constants for the pure culture growth are evaluated using cftool option in MATLAB 7.1 and are presented in Table 4 for *E .coli, S. aureus* (255) and *S. aureus* (261). From the correlation coefficient values (Table 4) it can be seen that the logistic model fits the pure culture growth of *E. coli* with reasonable accuracy for all the temperature conditions which can be viewed by the regression coefficients obtained ($R^2 > 0.95$ for all cases). The error percentages in all these cases are less than 7%. It can be inferred from the values of the logistic constants (Table 4) that, though β values did not change considerably, 'k' values show increasing trend with increasing temperature. The implication is that though the stationary phase numbers of the species did not change with temperature, the rate at which it is attained increases with the temperature. Similar observations are found with the *S. aureus* 255 and 261 in their pure culture growths. From the k values of the two species it can be seen that *E. coli* has higher growth rate. Hence the utilization of the substrate by this species is predominant than that by both the strains of *S.aureus*.

In mixed culture case the function $f_{12}$ is second order whereas $f_{21}$ is of third order which implies that the second species' growth is more declined than the second due to the competitive effect. It was inferred that the competitive effect is independent of temperature for the coliform, whereas for *S aureus* it is strongly dependent on temperature and the suppression is more at low and high temperatures (15 and 44 °C) than at 30 °C. The interaction parameters are found to vary with respect to temperature and are presented in Table 5. The theoretical growth values are evaluated by solving the differential equations (11) and (12) using MATLAB 7.1 software. The comparison between experimental and predicted growth rates for the two sets at various temperature conditions specified are represented in figures 4 to 9. The simulation study on chemostat model showed that continuous culture of these two systems is not possible.

**Table 4. Logistic constants and correlation coefficients for**
**_E .coli, S. aureus_ (255) and _S. aureus_ (261)**

| Species | Temperature (° C) | k (h⁻¹) | β (numbers⁻¹) |
|---|---|---|---|
| *E .coli* | 15 | 0.108 | $2.93 \times 10^{-9}$ |
| | 30 | 0.598 | $3.49 \times 10^{-9}$ |
| | 44 | 1.252 | $3.04 \times 10^{-9}$ |
| *S. aureus* (255) | 15 | 0.0194 | $3.58 \times 10^{-9}$ |
| | 30 | 0.1173 | $3.78 \times 10^{-9}$ |
| | 44 | 0.4275 | $3.24 \times 10^{-9}$ |
| *S. aureus* (261) | 15 | 0.0313 | $3.15 \times 10^{-9}$ |
| | 30 | 0.2083 | $3.62 \times 10^{-9}$ |
| | 44 | 1.002 | $3.45 \times 10^{-9}$ |

**Table 5: Interaction parameters for different temperatures**

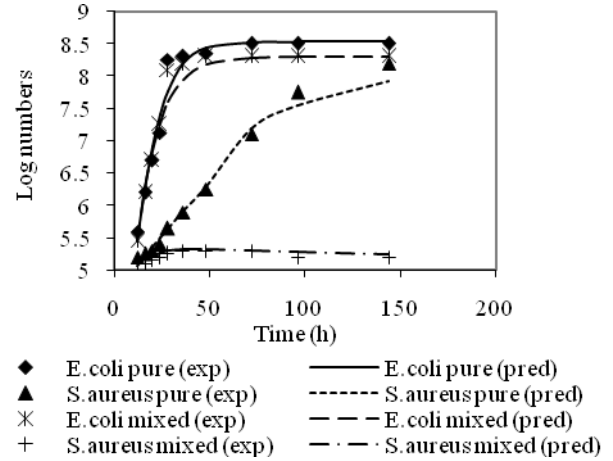| Temperature (°C) | *E.coli - S. aureus* 255 | | *E.coli - S. aureus* 261 | |
|---|---|---|---|---|
| | $a_{12}$ (1/number hour) | $a_{21}$ (1/number² hour) | $a_{12}$ (1/number hour) | $a_{21}$ (1/number² hour) |
| 15 | $9.13 \times 10^{-4}$ | $1.82 \times 10^{-3}$ | $6.49 \times 10^{-4}$ | $3.92 \times 10^{-3}$ |
| 30 | $8.76 \times 10^{-4}$ | $8.69 \times 10^{-4}$ | $6.12 \times 10^{-4}$ | $3.11 \times 10^{-3}$ |
| 44 | $7.64 \times 10^{-4}$ | $2.30 \times 10^{-3}$ | $5.72 \times 10^{-4}$ | $4.49 \times 10^{-3}$ |



**Fig.4 Experimental and predicted values of the growth of**
**_E.coli_ and _S. aureus_ 255 in pure and mixed culture at 15 ºC**
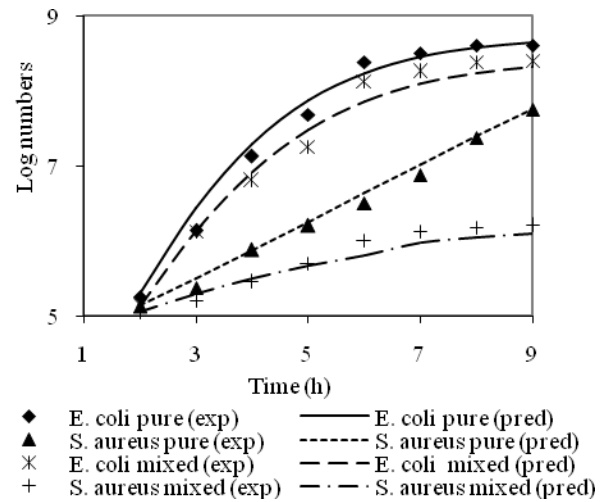


**Fig.5 Experimental and predicted values of the growth of**
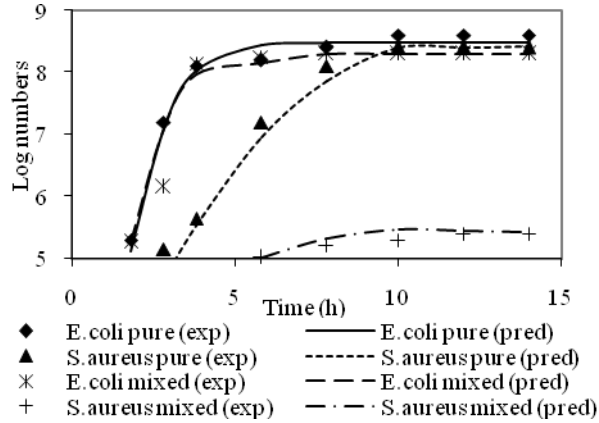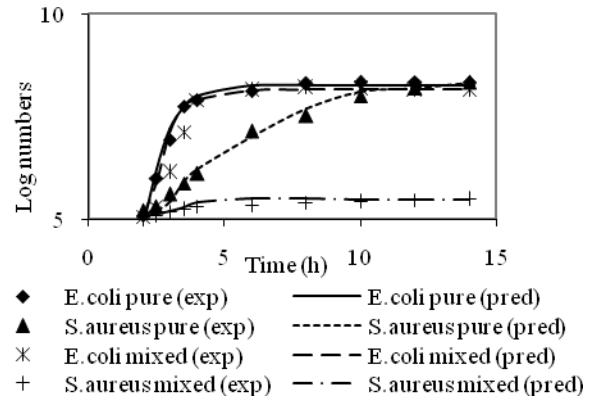**_E.coli_ and _S. aureus_ 255 in pure and mixed culture at 30 ºC**

**Fig.6 Experimental and predicted values of the growth of**
***E.coli*** **and** ***S. aureus* 255 in pure and mixed culture at 44 ºC**
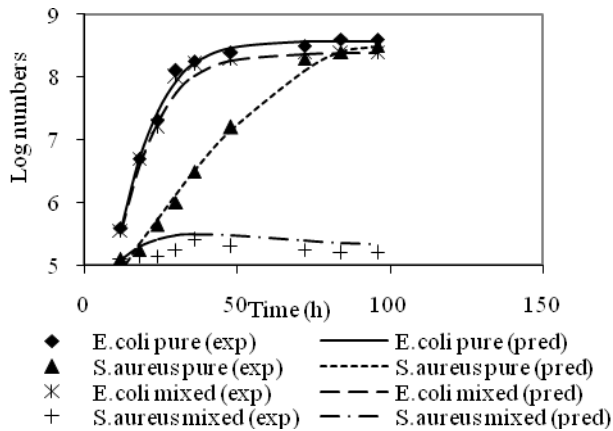


**Fig.7 Experimental and predicted values of the growth of**
***E.coli*** **and** ***S. aureus* 261 in pure and mixed culture at 15 ºC**



**Fig.8 Experimental and predicted values of the growth of**
***E.coli*** **and** ***S. aureus* 261 in pure and mixed culture at 30 ºC**
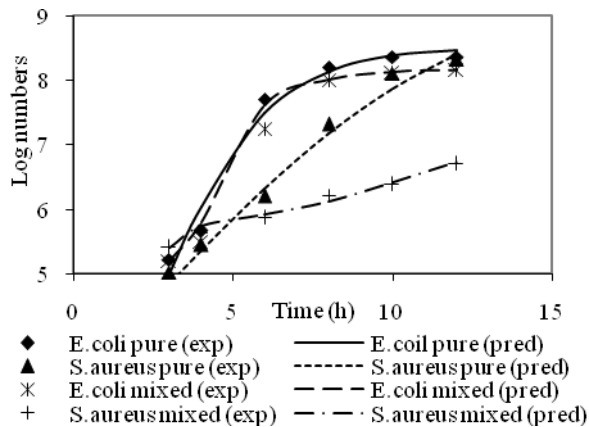


**Fig.9 Experimental and predicted values of the growth of**
***E.coli*** **and** ***S. aureus* 261 in pure and  mixed culture at 44 ºC**

## 3.3 Commensalism

System: *Streptococcus thermophilus* (1) and *Lactobacillus bulgaricus* (2)

Modeling studies of the commensal pattern of interaction for the system *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in batch case (D = 0) using the literature data [8] revealed that the interaction function for the species 1 by 2 is of first order.

$$\frac{dx_1}{dt} = -Dx_1 + k_1 x_1 (1 - \beta_1 x_1) + a_{12} x_2 \qquad (13)$$

$$\frac{dx_2}{dt} = -Dx_2 + k_2 x_2 (1 - \beta_2 x_2) \qquad (14)$$

These equations were able to fit the experimental growth with reasonable accuracy. The logistic constants along with the regression coefficients are given in Table 6. The interaction parameter $a_{12}$ is calculated as 10.281 (1/h).

**Table 6: Values of the logistic constants**

| Species | k (h⁻¹) | β (ml/numbers) | R² |
|---|---|---|---|
| *Streptococcus thermophilus* | 4.224 | $1.00 \times 10^{-8}$ | 0.9892 |
| *Lactobacillus bulgaricus* | 0.52 | $1.41 \times 10^{-9}$ | 0.9885 |

The simulation studies for continuous culture was carried out by solving equations (13) and (14) for steady state and given as equations (15) and (16).

$$x_{1s} = \frac{-(D-k_1) + \sqrt{(D-k_1)^2 + 4k_1\beta_1(a_{12}x_{2s})}}{2k_1\beta_1} \qquad (15)$$

$$x_{2s} = \left(1 - \frac{D}{k_2}\right)\frac{1}{\beta_2} \qquad (16)$$

These were solved using C++ software and the simulation result is given as surface plot (Fig.10) using MINITAB software package. The results indicate that the dilution rate in the chemostat should not exceed 0.52 h⁻¹ for coexistence of both the species. Also it was inferred that when the dilution rate is maintained below 0.26 h⁻¹ the yield of bacillus species is predominant and in the range 0.26 > D > 0.52 the coccal species' yield is predominant. Thus

when the species 1 or the product from it is desired, the chemostat should be operated in the dilution rate below 0.26 h$^{-1}$ and if the second species is desired, a dilution rate greater than 0.26 h$^{-1}$ (but lesser than 0.52 h$^{-1}$) should be maintained.
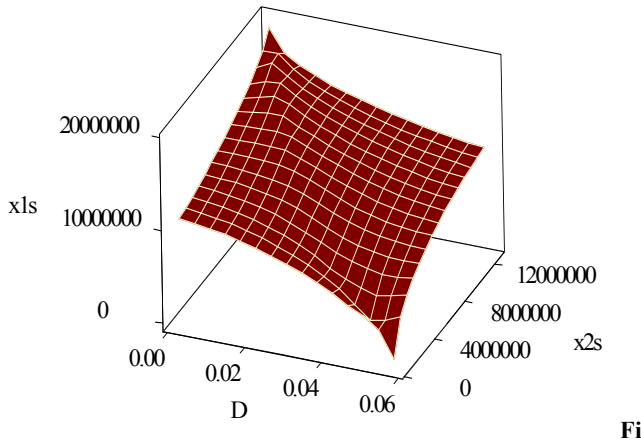


**Fig.10 Surface Plot of Dilution rate vs steady state concentrations of *S. thermophilus* and *L. bulgaricus* in mixed culture**

## 3.4 Mutualism

System: *Geotrichum candidum* (1) and *Penicillium camembertii* (2)

The mutualistic interaction between the two fungal populations *Geotrichum candidum* and *Penicillium camembertii* was modelled using the experimental data available from literature [2].

$$\frac{dx_1}{dt} = -D(x_1) + k_1 x_1 (1 - \beta_1 x_1) + a_{12} \frac{x_2}{x_1 + x_2} \qquad (17)$$

$$\frac{dx_2}{dt} = -D(x_2) + k_2 x_2 (1 - \beta_2 x_2) + a_{21} \frac{x_1}{x_1 + x_2} \qquad (18)$$

The interaction parameters $a_{12}$ and $a_{21}$ become zero for pure culture and D is zero for batch case in the above equations. The logistic constants and the regression coefficients are given in Table 7.

**Table 7. Parameters of the Logistic model**

| Species | Logistic constants | | $R^2$ |
|---|---|---|---|
| | k (1/h) | β (ml/cfu) | |
| *Geotrichum candidum* | 0.0572 | 5.01 x 10$^{-8}$ | 0.9932 |
| *Penicillium camembertii* | 0.0295 | 7.94 x 10$^{-8}$ | 0.9983 |

The mutualistic effect is more pronounced for the second species than the first. This is evident from the values of the interaction constants ($a_{12}$ = 400.34 cfu/ml h and $a_{21}$ = 4.5 x 10$^4$ cfu/ml h). The simulation studies are carried out by solving equations (13) and (14) for steady state conditions and the result are given as surface plot (Fig.11) obtained using MINITAB. It is inferred that that the former species shows relatively higher decrement with the increasing dilution rate when compared to the latter. The decrement trend is found to be sharp for the candidum species for the entire range of the dilution rates obtained from

simulation whereas for the penicillium species it is sharp till 0.03 h$^{-1}$ and beyond this it attains a minimum (non zero) value. It is observed from the simulation data that coexistence of both the species can be attained only when the dilution rate of the chemostat is maintained lesser than 0.0576 h$^{-1}$ above which *G. candidum* becomes extinct.
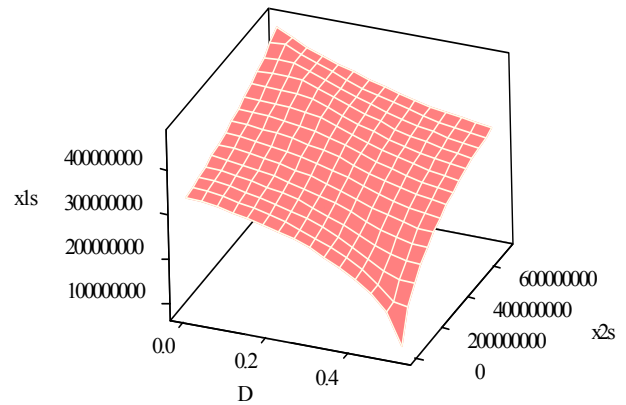


**Fig. 11 Surface Plot of Dilution rate vs steady state concentrations of *G. candidum* and *P. camembertii* in mixed culture**

## 4. CONCLUSIONS

The analysis of multiple interactive microbial populations is a fascinating area of research that involves mathematical modeling to quantify the several interactive effects in describing the growth rates of the interacting species in mixed culture. The present study deals with the development of an innovative and unified approach in describing the mixed microbial population kinetics for all the interaction patterns. During mixed culture operations the growth of all species depends not only on the environmental conditions and the available nutrients for each species but also on the interaction among them. In the present work the modeling study of four patterns of interaction has been carried out. Also the simulation studies were performed using MATLAB 7.1, C++ to obtain the stable operating conditions in continuous mode.

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