Kinetic Modelling of competitive interaction between *Escherichia coli* and *Staphylococcus aureus* at different temperatures

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Abstract
Competitive type of interaction among mixed microbial systems is more common in industrial applications. To attain coexistence of the various species growing together in a batch or continuous processes, the mechanism of interaction and appropriate models to define the mechanism is essential for the design and control of the biochemical reactions. In the present study, an attempt has been made to model the competitive interaction mechanism of Escherichia coli and Staphylococcus aureus at various temperature conditions.

The pure culture growths followed logistic model and the interaction effect for the coli species is found to be second order and the aureus species follows third order. The growth parameters and interaction coefficients were computed using MATLAB 7.1 software and presented. The simulation study for continuous process in a chemostat model revealed that coexistence is not possible.

Keywords: Kinetic modelling, interaction, *E. coli*, *S. aureus.*

Introduction

Most of the biochemical operations involve mixed culture operations on industrial scale. The performance of the processes depend on the interaction pattern.[1] Interactions can be classified as positive and negative type.[2-4] Microbial competition plays an important role in natural eco systems as well as industrial processes that employ mixed cultures[5]. Competition represents a negative relationship between two populations in which both populations are adversely affected with respect to their survival and growth[6].

The populations may achieve lower maximal densities or lower growth rates than they would have in the absence of competition. Competition occurs when two populations use the same resource, whether space or a limiting nutrient. Competition may occur for any growth-limiting resource. Available sources of carbon, nitrogen, phosphate, oxygen, iron growth factors, water and so on are all resources for which microbial populations may compete[4].

The development of dominant populations represents a case of competitive displacement. Abiotic parameters, such as temperature, pH and oxygen, greatly influence the intrinsic growth rates of microbial populations and the outcome of a competitive struggle.

A special type of competition is the pure and simple competition[7] in which there is only one nutrient whose availability affects the growth rates of the interacting populations. In the biochemical operations employing mixed culture systems, may it be in batch or continuous mode, the coexistence of all the interacting (or competing) species depends not only on their individual growth characteristics but also on the ability of them to utilize the available nutrients[8].

Such a kind of system can be attained in the laboratory in a chemostat that contains the microbial populations fed with the medium containing the limiting nutrient for the growth of the populations. In such a case, coexistence is predicted theoretically for discrete values of the chemostat dilution rate only when the curves of the specific growth rate as a function of limiting nutrient concentration cross[9].

The dilution rate must have exactly the value at which the specific growth rates of the population in the chemostat are equal[10]. With the above facts in background, the present research intends to model the competitive interaction for a specific system in batch case and extend them for continual process to predict the feasibility of stable operating conditions.

One of the major microorganisms present in food products is the *Staphylococcus aureus* and most of the strains of this species are enterotoxigenic. The frequency of outbreaks of staphylococcal food poisoning has stimulated interest in the factors that influence the growth of staphylococci in foods and the production of enterotoxin. One factor that has received comparatively little attention is the effect of simultaneous growth of other competing microorganisms.

This phenomenon can be regarded as of greater importance because the suppression of coccus species can be accomplished by inoculation of another species that can displace the available nutrients for coccal species without causing any damage to the food products. The choice of the second species lies on its inability to produce any toxic substances for the food products, resistant to adverse effects by the metabolic activity of the coccal species (if any) and should possess higher growth rate relatively.

According to Regnier and Lambin,[11] *Escherichia coli* is antagonistic towards *Staphylococcus aureus* in nutrient broth. A decrease in the inoculation level of *E. coli* species lessened the inhibition of the staphylococcus[12]. Similar trend was reported in different environmental condition of food products processing with this system by several other authors[13-15]. *E. coli* is a commonly occurring organism that does not produce appreciable amounts of antibiotic products and possesses higher growth rate when compared to the coccal organism[16]. Hence addition of this strain can be considered as a potential method of removing toxicity synthesized by staphylococcal strains.

The experiments by Oberhofer and Frazier[16] on pure and associated culture of *E. coli* and *S. aureus* include the investigations with two different strains of *S. aureus* (255 and 261) at three different temperatures (15, 30 and 44ºC) in meat infusion broth in batch mode. For the studies of competitive growth in broth, numbers of bacteria in this culture were counted by the direct microscopic method and enough of the culture was used as an inoculum to give 100,000 cells per ml in the inoculated broth tube. For the plate counts, mannitol salt agar was used for enumeration of viable staphylococci and violet red bile agar for *E. coli.*

Mathematical modelling: An attempt has been made to develop suitable models that fit the experimental data for these systems based on the general cell balance equations for two species (i and j) interacting in a common environment under batch conditions[17] given by equations (1) and (2):

$$\frac{dx_i}{dt} = a_{ii}x_i + a_{ij}f_{ij}(x_j, x_i) \quad (1)$$

$$\frac{dx_j}{dt} = a_{jj}x_j + a_{ji}f_{ji}(x_j, x_i) \quad (2)$$

The first term in the right hand side of these two equations representing the pure culture growth of each species was found to be well explained by the logistic model. The functions in the second term which account for the interactive effects were obtained by trying with different combinations. These are given as:

$$\frac{dx_1}{dt} = k_1x_1(1-\beta_1x_1) - a_{12}{x_2}^2 \quad (3)$$

$$\frac{dx_2}{dt} = k_2x_2(1-\beta_2x_2) - a_{21}{x_1}^2x_2 \quad (4)$$

For a chemostat these equations become:

$$\frac{dx_1}{dt} = -Dx_1 + k_1x_1(1-\beta_1x_1) - a_{12}{x_2}^2 \quad (5)$$

$$\frac{dx_2}{dt} = -Dx_2 + k_2x_2(1-\beta_2x_2) - a_{21}{x_1}^2x_2 \quad (6)$$

where x is the microbial abundance (numbers), k (per hour) and β (numbers^{-1}) are the logistic constants and the subscripts 1 and 2 refer to *E. coli* and *S. aureus* respectively. The constants a_{12} (1/number hour) and a_{21} (1/number^2 hour) are the interaction parameters. Under pure culture case, the interaction constants become zero. D is the dilution rate in per hour. For the sake of clarity the pair *E. coli* - *S. aureus* (255) will be denoted as set 1 and *E. coli* - *S. aureus* (261) as set 2 in our further discussions. The scheme of solution to the model equations and determination of parameters is performed using MATLAB 7.1 software.

Results and Discussion

Batch culture - Pure Culture: The model for pure culture is obtained from the equations (3) and (4) by making the interaction constants a_{12} and a_{21} zero. 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 1 for *E. coli*, *S. aureus* (255) and *S. aureus* (261). The comparison between experimental and predicted growth rates for the two sets at various temperature conditions specified is represented in figures 1 to 6.

From the correlation coefficient values (Table 1), 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%.

Table 1
Logistic constants and correlation coefficients for *E. coli, S. aureus* (255) and *S. aureus* (261):

Species	Temperature (ºC)	k (h^{-1})	β (numbers^{-1})	R^2
E. coli	15	0.108	2.93 x 10^{-9}	0.9635
	30	0.598	3.49 x 10^{-9}	0.9935
	44	1.252	3.04 x 10^{-9}	0.9889
S. aureus (255)	15	0.0194	3.58 x 10^{-9}	0.9838
	30	0.1173	3.78 x 10^{-9}	0.9949
	44	0.4275	3.24 x 10^{-9}	0.9906
S. aureus (261)	15	0.0313	3.15 x 10^{-9}	0.9913
	30	0.2083	3.62 x 10^{-9}	0.9897
	44	1.002	3.45 x 10^{-9}	0.9536

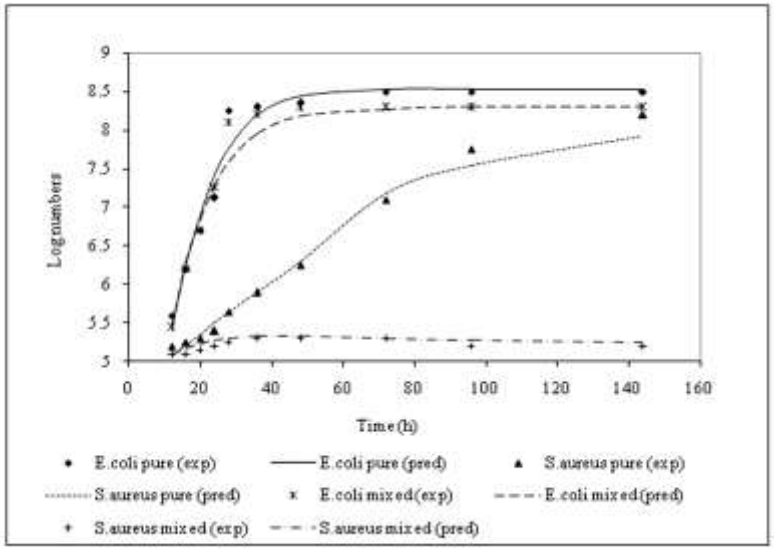

Fig. 1: Experimental and predicted values of the growth of *E. coli* and *S. aureus* 255 in pure and mixed culture at 15 ºC

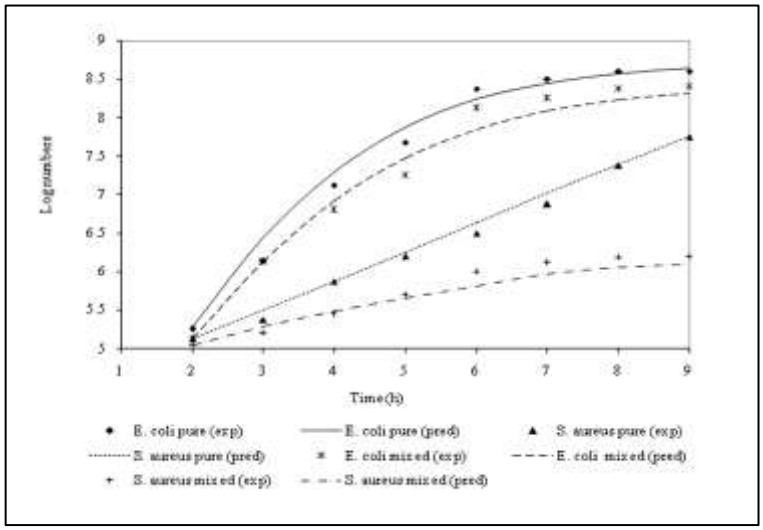

Fig. 2: Experimental and predicted values of the growth of *E. coli* and *S. aureus* 255 in pure and mixed culture at 30 ºC

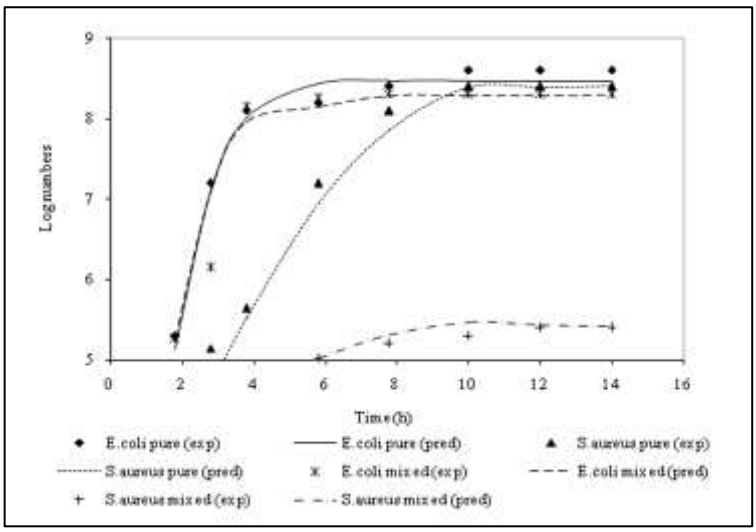

Fig. 3: Experimental and predicted values of the growth of *E. coli* and *S. aureus* 255 in pure and mixed culture at 44 ºC

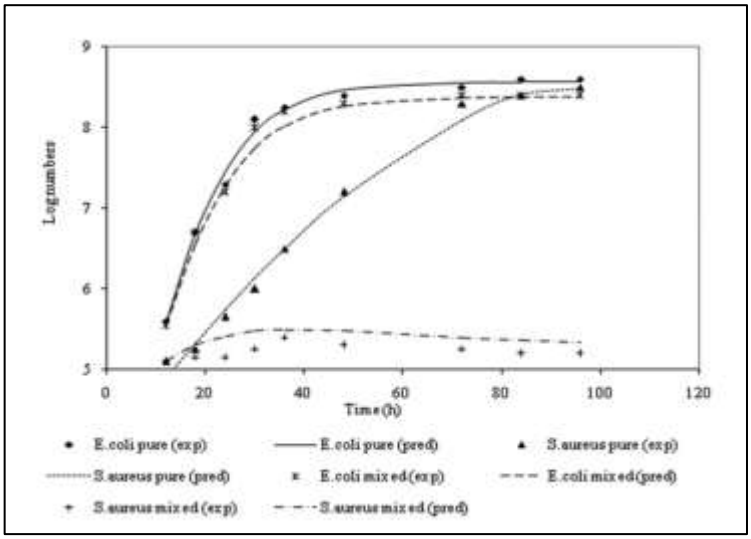

Fig. 4: Experimental and predicted values of the growth of *E. coli* and *S. aureus* 261 in pure and mixed culture at 15 ºC

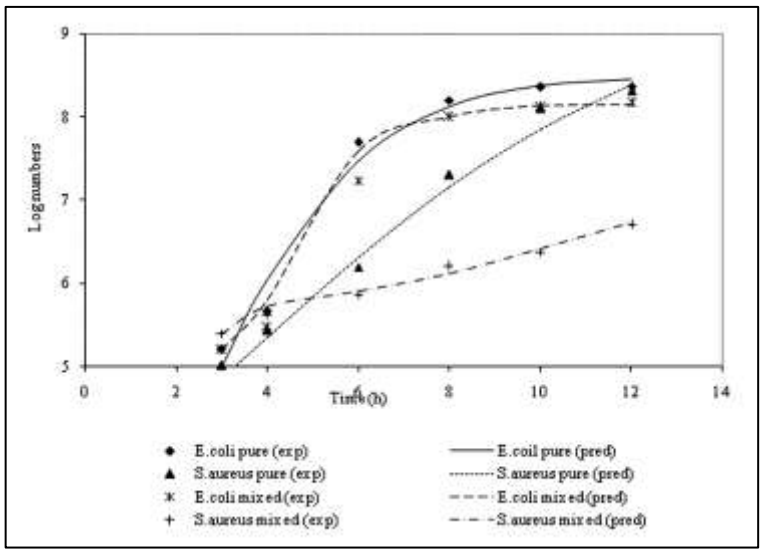

Fig. 5: Experimental and predicted values of the growth of *E. coli* and *S. aureus* 261 in pure and mixed culture at 30 ºC

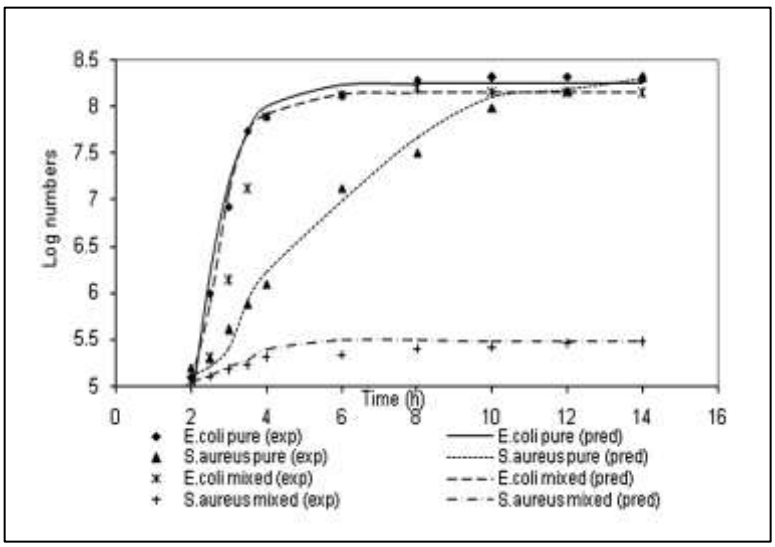

Fig. 6: Experimental and predicted values of the growth of *E. coli* and *S. aureus* 261 in pure and mixed culture at 44 ºC

It can be inferred from the values of the logistic constants (Table 7) 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.*

Effect of temperature on growth constants: The maximum growth for the *E. coli* species is attained in 11 h itself at 30 ºC and a bit earlier at 40 ºC, whereas at a temperature of 15 ºC, the log phase growth commences only after 12 h and the stationary growth is attained at around 20 h. This can be viewed from the fig. 7. The same pattern of growth trend is observed for the *S. aureus* 255 and 261 (Figures 8 and 9). In these two cases the log phase growth is initiated after 12 h of inoculation and the maximum growth for both the strains is attained only after 100 h at 15 ºC. But at higher temperatures (30 and 44 ºC), the growth is found to be rapid and the maximum growth is reached in 10 h itself.

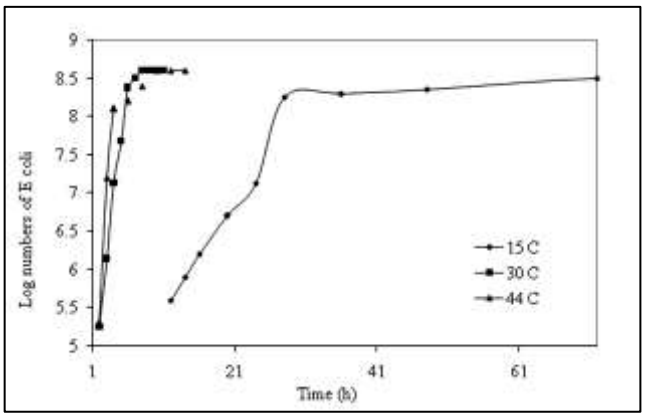

Fig. 7: Time Vs growth of *E. Coli* for different temperatures

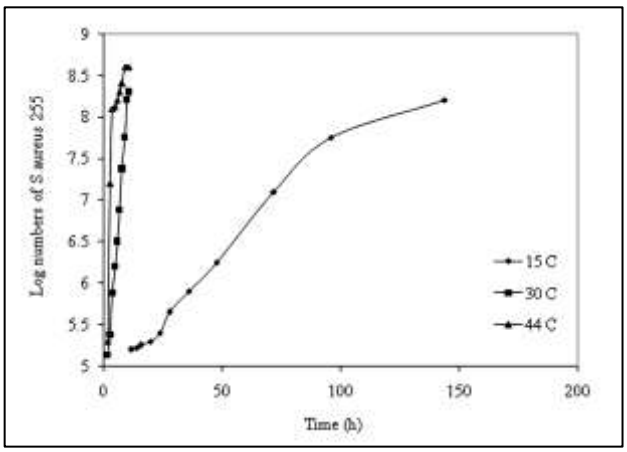

Fig. 8: Time vs growth of *S. aureus* 255 for different temperatures

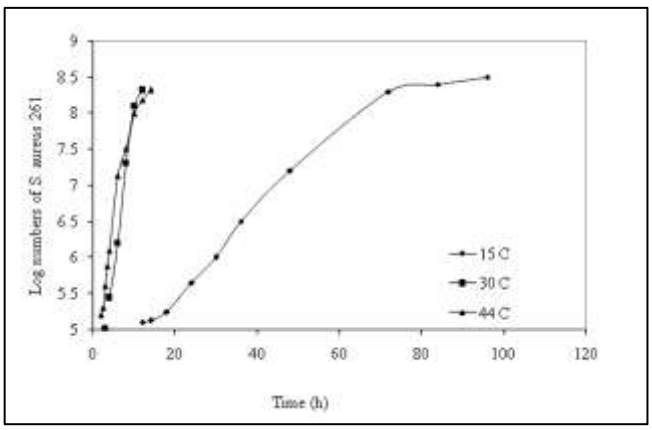

Fig. 9: Time vs growth of *S. aureus* 261 for different temperature

Model for temperature dependence: The temperature dependence of k is found to follow the Arrhenius type relation given as follows for all the species considered here with minimum error.

$$k = k_0 e^{-E'/T} \quad (7)$$

where k_0 (h^{-1}) and E' ($^oC^{-1}$) are constants. The term k_0 (frequency factor) can be considered as a measure of the frequency of the interaction between substrate and species that produces effective growth and E' (energy barrier) represents the resistance level for the growth. The values of these constants for the three species evaluated using cftool in MATLAB 7.1 software are given in table 2. The comparison between the actual and predicted k versus T data for the three species is portrayed in figure 10.

The mixed culture data for the two sets show that the interaction between the coliform and coccus organisms is of negative type since both the species experienced relatively lesser growth than the pure culture conditions. As mentioned earlier, the substrate utilization rate by coliform species is greater than that by coccal strains which is evident from the higher k values of the former species than the latter. Since there is no evidence for release of toxic substances by either of the species to other, it can be concluded that the competition for the substrate between the two species is the reason for the decelerated growth of them in associated growth.

The *E. coli*'s growth was less affected when compared to *S. aureus* 255 and 261 in all the three temperature conditions (Figure 11). The ratio of mixed to pure culture growth for the coliform species lies in the range of 0.9651 to 0.9784 in these temperatures whereas for the coccal organism, this ratio is relatively low indicating stronger suppression. The ratios are 0.6341, 0.7470 and 0.6429 for *S. aureus* 255 and that for *S. aureus* 266 are 0.6118, 0.8245 and 0.6587 at temperatures 15, 30 and 44^o C respectively.

The model for associated culture is found to fit the experimental data with greater accuracy for both the sets in all the temperature range studied. Fig. 1 shows the comparison of the experimental data with the values obtained from model equations represented by equations (2) and (3) for the set 1 operating at 15 ° C. It can be seen from the plot that the model is able to predict the growth rates for all the cases in a better manner. The error percentages in each case are within 9 %.

In figure 2 the results obtained for the set 1 at 30 ºC are presented along with the experimental data. In this condition the suppression of coccus species is about 10 times lesser when compared to that at 15 ºC. The model equations are found to fit the experimental data for this condition reasonably well with an error percentage within 6%. The level of inhibition of *S. aureus* 255 at 44 ºC is found to be 1.6 times lesser than at 15 ºC and nearly 6.3 times more than that at 30 ºC. The model fits the experimental values for the set 1 at 44 ºC too with higher accuracy (error percentage < 10%).

Table 2
Arrhenius type constants for *E. coli, S. aureus* (255) and *S. aureus* (261)

Species	k_o (h^{-1})	E' ($^oC^{-1}$)	R^2
E. coli	5.146	63.58	0.9936
S. aureus (255)	6.661	120.9	0.9967
S. aureus (261)	28.74	147.7	0.9983

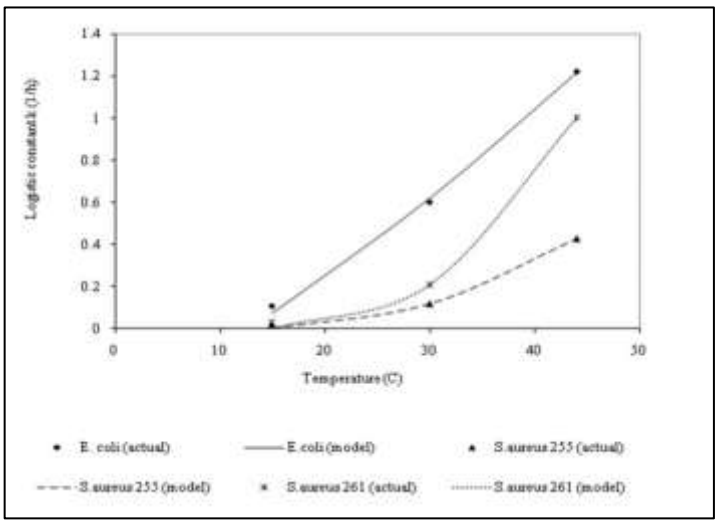

Fig. 10: Temperature Vs Logistic constant (k) Associated culture – Competition

Similar trend is observed for the set 2 for all the specified temperatures. The predicted model is found to fit the experimental data with minimum percentages of error (< 9%) for all the temperature conditions. The comparison between experimental and theoretical data is portrayed in figures 4, 5 and 6 for the temperatures 15, 30 and 44 ºC respectively. The decreasing effect is found to be more for the second species which can be viewed from the equations (3) and (4) for mixed culture growth of the two organisms.

The second term in these equations accounts for the interaction effect where the inhibition in *E. coli*'s growth varies as the square of the coccus species' concentration but that of *S. aureus* varies as a cubic function (power of two for *E.coli* and power of one for *S. aureus*). The interaction parameters for the two sets for the three temperature conditions are given in table 3.

It can be readily ascertained that the suppression level 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 ºC) than at 30 ºC. It can be seen from figure 12 that the interaction constant a_{12} is nearly the same for all the temperatures in both the sets.

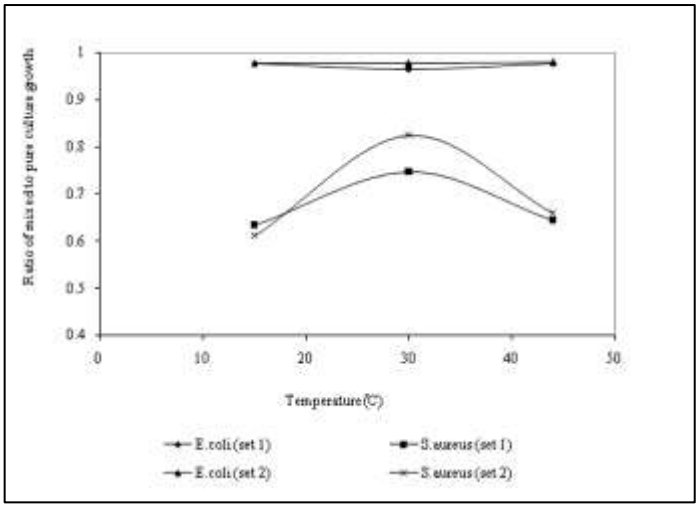

Fig. 11: Ratio of mixed to pure culture growth as a function of temperature

Table 3
Interaction parameters for different temperatures

Temperature (ºC)	***E. coli* - *S. aureus* 255**		***E. coli* - *S. aureus* 261**	
	a_{12} (1/number hour)	**a_{21}** (1/number^2 hour)	**a_{12}** (1/number hour)	**a_{21}** (1/number^2 hour)
15	9.13×10^{-4}	1.82×10^{-3}	6.49×10^{-4}	3.92×10^{-3}
30	8.76×10^{-4}	8.69×10^{-4}	6.12×10^{-4}	3.11×10^{-3}
44	7.64×10^{-4}	2.30×10^{-3}	5.72×10^{-4}	4.49×10^{-3}

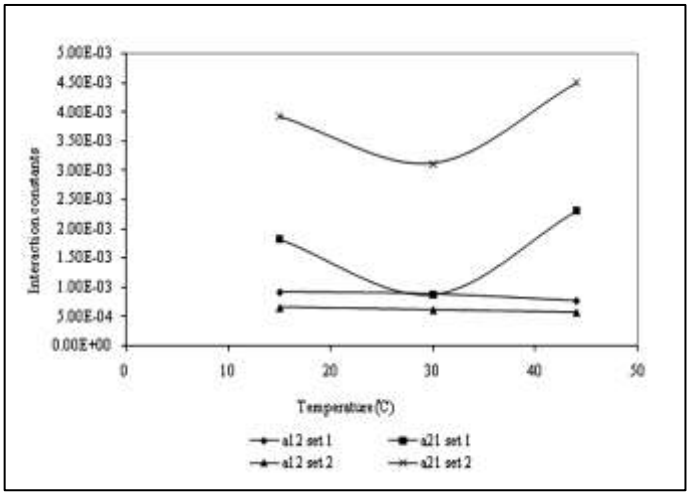

Fig. 12: Temperature vs Interaction constants

The equations (5) and (6) represent the chemostat model equations for the two species competing for their nutrients in the same environment. These two equations can be used to simulate the dilution rate to obtain stable operating conditions at which both the species can coexist. This was carried out by running a C++ program to solve these two equations.

The simulated data shows that only negative dilution rates can be obtained which is not physically possible in a real chemostat. Hence this system can be handled only in batch mode and a continual operation is not possible for coexistence of both the species.

Conclusion

The competitive interaction of the systems *E. coli* and *S. aureus* 255 and *E. coli* and *S. aureus* 261 based on the literature data for three different temperatures viz. 15, 30 and 44 ºC were modelled and the function f_{12} is second order whereas f_{21} is of third order. This is an implication that the second species' growth is more declined than the second due to the competitive effect.

Also, 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 44º C) than at 30º C. The simulation study on chemostat model showed that continuous culture of these two systems is not possible.

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(Received 05th October 2020, accepted 06th December 2020)