Modelling of interaction between Schizosaccharomyces pombe and Saccharomyces cerevisiae to predict stable operating conditions in a chemostat

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Abstract: In natural eco systems and industrial operations involving mixed culture systems, interactions among various species greatly influence the performance of the entire bioprocess. The study of the interaction patterns and modelling the process based on it will be of ample importance to obtain optimum operating conditions. Ammensalism is a kind of negative interaction that occurs among diverse microbial populations. Such kind of interaction exists between the yeasts Schizosaccharomyces and Saccharomyces cerevisiae when they are grown in a common environment with glucose and malic acid as the growth limiting substrates for both the species. In the present study the growth rates of both the species were tested for the validity of the logistic model under control culture operation. The logistic constants for both the species growing with two growth rate limiting substrates are reported. In addition suitable model that accounts for the interaction effects are incorporated in the growth rate equations and compared with the experimental growth rate values. The modelling equations are utilized to estimate the dilution rate to obtain stable operating conditions in a chemostat.

Keywords: Demalication, Chemostat, amensalism, logistic, dilution rate

Introduction

Distinct microbial populations frequently interact with each other in mixed culture operations. The performance and behaviour of the mixed culture systems greatly depends on the interaction patterns among the various species involved. Among the interaction patterns identified so far amensalism is one of the commonly occurring phenomena. Microorganisms that produce substances toxic to competing populations will naturally have a competitive advantage. The first population may be unaffected by the inhibitory substance or may gain a competitive edge that is beneficial. When one microbial population produces a substance that is inhibitory to other populations, the inter population relationship is called amensalism.

In this kind of interaction the second species gets negative effect due to the presence of the first species whereas the growth or survival of the former species is unaltered. This characteristic relation has been explained by many mechanisms including competition for substrates and inhibitory actions by the first on second. The terms antibiosis and allelopathy have been used to describe such cases of chemical inhibition. There are cases of complex amensalism between populations in natural habitats, for example, virucidal (virus-killing) factors in seawater and fungistatic (fungi-inhibiting) factors in soil. The basis of these relationships is believed to be amensalism, since sterilization eliminates the inhibitory factors, but the chemical background of these complex antimicrobial activities remains to be explored.

Ammensalism may lead to the pre-emptive colonization of a habitat. Once an organism establishes itself within a habitat it may prevent other populations from surviving in that habitat. The production of lactic acid or similar low-molecular-weight fatty acids is inhibitory to many bacterial populations. Populations able to produce and tolerate high concentrations of lactic acids, for example, are able to modify the habitat so as to preclude the growth of other bacterial populations. E. coli is unable to grow in the rumen, probably because of the presence of volatile fatty acids produced there by anaerobic heterotrophic microbial populations. Fatty acids produced by microorganisms on skin surfaces are believed to prevent the colonization of these habitats by other microorganisms. Populations of yeasts on skin surfaces are maintained in low numbers by microbial populations producing fatty acids. Acids produced by microbial populations in the vaginal tract are probably responsible for preventing infection by pathogens such as Candida albicans.
In wine manufacture removal of malic acid is a major concern which is accomplished using the yeasts *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* and it has been observed that the interaction\(^6\) between these two species is amensalistic. In the report given by them, only experimental data have been presented and modelling of the growth in pure and mixed culture conditions have not been discussed. Objectives of the present study are (i) to predict the kinetic parameters for the growth rate of *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* in pure culture in batch mode with two growth rate limiting substrates (glucose and malic acid), (ii) to predict suitable equations that account the interaction effects between them under same conditions and (iii) to extend the model to chemostat and estimate the dilution rates for stable operating conditions. The experimental data for the model development have been obtained from the reports of Taillandier et al\(^7,8,9,10\).

**Description of the system**

Malic acid is one of the two major acids of grape musts and its removal from wines is often necessary. *Schizosaccharomyces* yeasts are microorganisms naturally occurring in grape musts flora but not developing in usual wine-making conditions. The species *pombe* is able to metabolize malic acid to ethanol with a high efficiency and, therefore, can be used for biological deacidification of wines instead of the usual method that is malo-lactic fermentation (MLF)\(^7,8,9,10\). In order to produce high quality wine it is recommended that *Schizosaccharomyces pombe* is used for demalication and *Saccharomyces cerevisiae* for alcohol formation in a second step\(^10,11\). When only partial deacidification is desired for organoleptic reasons the action of *Schizosaccharomyces* yeasts has to be limited\(^12\). One way of limiting is to perform partial demalication by *Schizosaccharomyces pombe* and alcohol formation by *S. cerevisiae*, and to try to control the extent of deacidification\(^13\) by *Schizosaccharomyces pombe* without removing it from the medium by inoculating *S. cerevisiae* at different delayed time intervals.

**Mathematical model for mixed culture**

The general cell balance equations for two species (i and j) interacting in a common environment in a chemostat for a sterile medium are given by the equations

\[
\frac{dx_i}{dt} = -D(x_i) + a_ix_i + a_jx_j \tag{1}
\]

\[
\frac{dx_j}{dt} = -D(x_j) + a_jx_i + a_ix_j \tag{2}
\]

where \(x_i\) and \(x_j\) are the cell mass of species i and j respectively. The terms \(a_i\) and \(a_j\) represent the pure species growth rates of species i and j respectively. The constants \(a_i\) and \(a_j\) are the parameters that account for the interaction effects and \(D\) is the dilution rate. In batch culture conditions \(D\) becomes zero.

All possible combinations of interactions namely neutralism, mutualism, commensalism, amensalism, competition, predation and parasitism defined in this manner are shown in Table 1.

The experimental data were tested for its fitness to Monod’s model but it was found that both the species’ growth did not follow it. Most of the microbial growth kinetics can be described well with the logistic model with reasonable accuracy. Hence the logistic equation has been used in this investigation to predict the growth rate of the two species *Schizosaccharomyces pombe* (species 1) and *Saccharomyces cerevisiae* (species 2). As the interaction between the two species has been reported\(^6\) as amensalistic the term \(a_{ij}\) becomes zero and \(a_{21}\) becomes negative. It has been found that the change in the growth rate of the two species 2 is not a linear function of \(x_1\) and \(x_2\).

The modelling equations for batch and chemostat models for this system incorporating the interaction effects in terms of \(a_{21}\) are given below:

**Batch:**

\[
\frac{dx_1}{dt} = k_1x_1(1-\beta_1x_1) \tag{3}
\]

\[
\frac{dx_2}{dt} = k_2x_2(1-\beta_2x_2) + a_{21}\frac{x_1x_2}{1+x_1x_2} \tag{4}
\]

**Chemostat:**

\[
\frac{dx_1}{dt} = -D(x_1) + k_1x_1(1-\beta_1x_1) \tag{5}
\]

\[
\frac{dx_2}{dt} = -D(x_2) + k_2x_2(1-\beta_2x_2) + a_{21}\frac{x_1x_2}{1+x_1x_2} \tag{6}
\]

where \(x_1\) and \(x_2\) are the microbial abundance (millions ml\(^{-1}\)) of species 1 and 2 respectively. \(k_1, k_2, \beta_1\) and \(\beta_2\) are the constants of the logistic model for species 1 and 2 respectively. For pure species growth \(a_{12}\) and \(a_{21}\) becomes zero. Under steady state conditions the derivatives become zero and the resulting equations can be solved to estimate the dilution rate for the desired cell mass concentrations at steady state. It has been observed from the stability analysis that the necessary condition for stable operating condition is that \(D > k_1(1-\beta_1x_1)\) and \(k_2(1-\beta_2x_2)\).
Table 1. Classification of pair wise interactions based on the sign of the entries $a_{ij}$ and $a_{ji}$ ($i \neq j$)

<table>
<thead>
<tr>
<th>effect of species $j$ on species $i$ (sign of $a_{ij}$)</th>
<th>$-$</th>
<th>0</th>
<th>$+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>effect of species $i$ on species $j$ (sign of $a_{ji}$)</td>
<td>$-$</td>
<td>-- competition</td>
<td>$-$0 amensalism</td>
</tr>
<tr>
<td>0</td>
<td>0-amensalism</td>
<td>00 neutralism</td>
<td>0+commensalism</td>
</tr>
<tr>
<td>$+$</td>
<td>$+$-predation</td>
<td>$+$0commensalism</td>
<td>++mutualism</td>
</tr>
</tbody>
</table>

Table 2. Experimental growth data of *Schizosaccharomyces pombe and Saccharomyces cerevisiae* in control culture:

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Number of cells of <em>Schizosaccharomyces pombe</em> $x_1$ (millions/ml)</th>
<th>Time (h)</th>
<th>Number of cells of <em>Saccharomyces cerevisiae</em> $x_2$ (millions/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>13.5</td>
<td>15</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>16.5</td>
<td>20</td>
<td>15</td>
<td>75</td>
</tr>
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<td>18.5</td>
<td>25</td>
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<td>45</td>
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<tr>
<td>36.5</td>
<td>70</td>
<td>50</td>
<td>265</td>
</tr>
<tr>
<td>40</td>
<td>75</td>
<td>55</td>
<td>265</td>
</tr>
<tr>
<td>46</td>
<td>80</td>
<td>60</td>
<td>265</td>
</tr>
</tbody>
</table>

Table 3: Parameters of the Logistic model and interaction coefficient

<table>
<thead>
<tr>
<th><em>Schizosaccharomyces pombe</em></th>
<th><em>Saccharomyces cerevisiae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1$: 0.1259 h$^{-1}$</td>
<td>$k_2$: 0.2105 h$^{-1}$</td>
</tr>
<tr>
<td>$\beta_1$: 0.0125 ml millions$^{-1}$</td>
<td>$\beta_2$: 3.77 x 10$^3$ ml millions$^{-1}$</td>
</tr>
<tr>
<td>$a_{12}$: 0</td>
<td>$a_{21}$: -4.009 h$^{-1}$</td>
</tr>
</tbody>
</table>
Fig. 1 Growth curve for Species 1 (control culture)

Fig. 2 Growth curve for species 2 (control culture)

Fig. 3 Growth curve for species 1 in mixed culture in batch conditions

Fig. 4 Growth curve for species 2 in mixed culture in batch conditions

Fig. 5 Dilution rate vs steady state concentrations of S. pombe and S. cerevisiae
Fig. 6 Dilution rate vs steady state concentrations of S. pombe and S. cerevisiae

Fig. 7 Dilution rate vs steady state concentrations of S. pombe and S. cerevisiae

Fig. 8 Unsteady state profile of microbial concentration for $D = 0.02411$ per h
Results and Discussions

The kinetic data for the growth of the two species in control culture reported by Taillandier et al. is presented in Table 2. The values predicted from the logistic model according to equations (3) and (4) are found to fit the experimental data with minimum error (5.37 % for species 1 and 4.86% for species 2) which are represented in figures 1 and 2. In this case the interaction parameters $a_{12}$ and $a_{21}$ are zero. The logistic constants for both the species are reported in Table 3.

In the case of Saccharomyces cerevisiae the maximum specific growth was 0.29 per hour in the pure culture whereas it decreased to 0.21 per hour during mixed culture. The model represented in equation (4) proves its validity to represent the experimental data for the growth of this species in mixed culture with an error of 8.11% and regression coefficient of 0.97. The amensalistic behaviour of this system is well explained from the values of the interaction parameters $a_{12}$ (≈0) and $a_{21}$ (≈ -4.009 h$^{-1}$).

In the design calculation for chemostat using equations (5) and (6) a definite range of steady state values for the cell mass concentrations $x_1$ and $x_2$ for the species 1 and 2 respectively were fixed satisfying the stability conditions. Then the dilution rate required for each set of the steady state concentrations were evaluated using C program. These stable operating points are represented in the figures 5 to 7 wherein the steady state concentrations of the two species are plotted against the dilution rate.

It can be inferred from these figures that the dilution rate has significant influence on the steady state growth of both the species. The former species shows relatively higher decrement with the increasing dilution rate when compared to the latter. In the dilution rate between 0.094 and 0.0017 h$^{-1}$ the steady state cell microbial abundance for species 1 decreased by 74.5 % whereas the second species experienced a decrement of 45.3 %. For each set in the stable region the unsteady state profiles of the colony forming units of both the species were obtained by solving the differential equations using numerical integration technique in MATLAB 7.1 platform. Fig. 8 is the pictorial representation of the unsteady growth of the two species for the dilution rate of 0.0241 h$^{-1}$. In this state the $x_{1s}$ and $x_{2s}$ values are 64.6954 millions ml$^{-1}$ and 232.7314 millions ml$^{-1}$.

Conclusions

The interaction pattern between Schizosaccharomyces pombe and Saccharomyces cerevisiae has been analysed quantitatively for amensalistic relation. It has been observed that the growth pattern of both the species follow logistic model with minimum error for the control culture cases. The mixed culture growth pattern also follows the logistic pattern for Schizosaccharomyces and the modified logistic model incorporating the interaction effect of this species with Saccharomyces cerevisiae too describes the experimental growth with minimal error. The chemostat model equations were solved to simulate the dilution rate values to obtain desired steady state microbial abundance for both the species under stable operating conditions.

References

4. Pohunek M., Streptococci antagonizing the vaginal Lactobacillus., Journal of Hygiene, Epidemiology, Microbiology and Immunology, 1961, 5, 267.