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Simulation of Increasing the Cell Membrane Permeability Under Steady – State Conditions

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ABSTRACT

There is being presented simulation of increasing membrane permeability cellular baker's yeast for the set values concentrations of alcohol solutions. It allows for easy activity forecasting intracellular catalase. Due to the costs associated with disposal large volumes of waste and the purchase of permeabilizing agents the tolerance of the permeabilization process to changes in alcohols concentration was checked. For this purpose, appropriate mathematical models were used. The obtained results confirm the high sensitivity of the process to changes in concentration permeabilizing factor. Of the three permeabilizations studied, this is the process using ethanol turned out to be the least tolerant to change concentration of the permeabilizing factor. The concentration of the solution of this alcohol, which will be used in permeabilization to obtain cells yeast with catalase activity half of the optimal activity, is only 20% lower than the optimal concentration. Processes permeabilization using the other two alcohols considered less sensitive to changes in their concentration. The concentrations of 1-propanol and 2-propanol solutions for $A^S = ½\ A^{opt}$ are 2.5 times and 3.5 times lower than the optimal concentrations, respectively.

Keywords: simulations, computer modeling, permeabilization, catalase, optimization, *Saccharomyces cerevisiae*.

INTRODUCTION

Simulations and computer modeling, thanks to its usefulness, is helpful in many areas of science and industry [Zhou, 2013; Winsberg, 2015; Mityushev et al., 2018]. They enable foreseeable scenarios and situations that can impact the environment, thus allowing actions to be taken to avoid them [Luo et al., 2021]. The development of biotechnology, which has allowed for the widespread use of microorganisms in industry, also finds its reflection in improved environmental protection [Skliar et al., 2020; Ugya and Meguellati, 2022]. One of the most commonly used yeasts is Saccharomyces cerevisiae [Willaert, 2017]. Many biotransformations using their intracellular enzymes are known [Lane et al., 2018; Ogra et al., 2018; Pandebesie et al., 2019]. This yeast is a rich source of catalase – one of the most industrially significant enzyme inview of the ability to degrade H_2O_2 into water and oxygen [Nishimoto et al., 2016; Kaushal et al., 2018]. The use of whole yeast cells with the enzyme remaining inside them is difficult due to the low permeability of the cell membrane [Trawczyńska 2020]. Limiting the diffusion of substrates and products results in low catalytic activity of cells. However, the isolation of the enzyme from inside the cells involves expensive purification procedures and partial deactivation, and the disadvantage of the isolated enzyme is that it cannot be reused multiple times [Mukherjee, 2019; Liu and Kokare, 2023]. An alternative solution to this problem is to use biocatalysts in the form of whole yeast cells with increased permeability as an enzyme source. One of the techniques for modifying the cytoplasmic membrane is permeabilization [Rasmussen et al., 2016; Santos et al., 2018; Chan et al., 2021]. Under the influence of the permeabilizing agent, the structure of the cell membrane is changed so that the pores that form enable the free exchange of molecules such as substrate or product [Zhao et

al., 2014; Gravel et al., 2017]. Permeabilized cells used for catalytic processes can be easily separated from the reaction environment and reused without the major loss of activity. According to one of the assumptions of the Zero Waste theory, when designing and managing products and processes, the volume of waste should be systematically reduced and all resources should be processed and recovered [Hamid et al., 2020]. In this work, based on appropriate mathematical models [Trawczyńska et al., 2018], an increase in the permeability of cell membranes of wet baker's yeast was simulated for set concentration values of alcohol solutions in order to enable easy prediction of intracellular catalase activity. This action is aimed at reducing the consumption of substrates during the permeabilization process and, consequently, also waste.

Mathematical models

Mathematical models used to simulate the increase in cell membrane permeability were formulated based on the Response Surface Methodology rules (Table 1) [Trawczyńska et al., 2018]. They permitted evaluation of linear, quadratic and interactive terms of the independent variables (temperature T, concentration of alcohol S and process times t) on the dependent variable (catalase activity A). The process was simulated for the appropriate measurement ranges T, S and t (Table 2). Based on mathematical models, using numerical techniques and the Mathcad 15 computer program, the values of the process parameters at which the catalase activity is the highest A^{opt} were calculated. Carrying out the permeabilization process of baker's yeast cells using 2-propanol allows achieving the highest enzymatic activity = 6310 $U \cdot g^{-1}$. For permeabilization with ethanol and 1-propanol, the optimal enzyme activity is = 5070 $U \cdot g^{-1}$ and = 4310 $U \cdot g^{-1}$, respectively.

ANALYTICAL SOLUTION

Depending on the requirements, the process optimization criterion can be defined in different ways. A common assumption is the lowest consumption of substrates, therefore the effectiveness of permeabilization with alcohols was tested for fixed concentrations of their solutions. Assuming S = const, the number of unknowns in the mathematical models decreases to two, thanks to which the T and t values and the corresponding A^S (maximum catalase activity) can be calculated using analytical methods. The determined first-order partial derivatives with respect to T and t for the mathematical models used are presented in Table 3. Equating the determined derivatives to zero allowed for the creation of an appropriate system of equations. Its solution are stationary points with coordinates (T, t) suspected of being extremum. The final formulas for calculating the temperature and duration of the permeabilization process carried out for the imposed concentrations are given

Table 1. Mathematical models

Permeabilising agent	Mathematical model
Ethanol	$A_E = 4919 + 545 \cdot S_E + 464 \cdot t_E - 1574 \cdot T_E^2 - 1405 \cdot S_E^2 - 570 \cdot t_E^2 - 512 \cdot T_E \cdot S_E$
1-Propanol	$A_{1P} = 4116 + 851 \cdot S_{1P} - 1032\ T_{1P}^2 - 1059 \cdot S_{1P}^2 - 386 \cdot t_{1P}^2 - 378 \cdot T_{1P} \cdot S_{1P} - 358 \cdot S_{1P} \cdot t_{1P}$
2-Propanol	$A_{2P} = 5784 + 477 \cdot T_{2P} + 1327 \cdot S_{2P} - 1343\ T_{2P}^2 - 960 \cdot S_{2P}^2 - 473 \cdot t_{2P}^2 - 675 \cdot T_{2P} \cdot S_{2P} - 484 \cdot S_{2P} \cdot t_{2P}$

Table 2. Measuring ranges and estimate optimal values of permeabilization parameters

Permeabilising agent	Measuring ranges	Temperature (T), °C	Alcohol concentration (S), %	Time (t), min
Ethanol	Minimal	6.6	24.8	7
	Maximal	23.4	75.2	73
	Optimal	14.9	52.6	48
1-Propanol	Minimal	1.5	3.2	7
	Maximal	18.5	36.8	73
	Optimal	10.3	24.2	41
2-Propanol	Minimal	6.6	6.4	7
	Maximal	23.4	73.6	73
	Optimal	15	55	40

Table 3. First-order partial derivatives and formulas for calculating the values *T* and *t*

Permeabilising agent	First-order partial derivatives	Formulas for calculating the values T and t
Ethanol	$\frac{dA_E}{dT_E} = -3148 \cdot T_E - 512 \cdot S_E$	$T_E = -0{,}1626 \cdot S_E$
	$\frac{dA_E}{dt_E} = 464 - 1140 \cdot t_E$	$t_E = 0{,}407$
1-Propanol	$\frac{dA_{!P}}{dT_{1P}} = -2064 \cdot T_{1P} - 378 \cdot S_{1P}$	$T_{1P} = -0{,}1831 \cdot S_{1P}$
	$\frac{dA_{!P}}{dt_{1P}} = -772 \cdot t_{1P} - 358 \cdot S_{1P}$	$t_{1P} = -0{,}4637 \cdot S_{1P}$
2-Propanol	$\frac{dA_{2P}}{dT_{2P}} = 477 - 2686 \cdot T_{2P} - 675 \cdot S_{2P}$	$T_{2P} = 0{,}1775 - 0{,}2513 \cdot S_{2P}$
	$\frac{dA_{2P}}{dt_{2P}} = -946 \cdot t_{2P} - 484 \cdot S_{2P}$	$t_{2P} = -0{,}5116 \cdot S_{2P}$

in Table 3. Then, second-order derivatives were determined and appropriate determinants were created in accordance with the equation.

$$W = \begin{vmatrix} \frac{d^2A}{dT^2} & \frac{d^2A}{dTdt} \\ \frac{d^2A}{dTdt} & \frac{^2A}{dt^2} \end{vmatrix} \quad (1)$$

The determinants for stationary points were calculated and on their basis it was checked whether there is an extremum in the points (W > 0). Then, based on the values of second-order derivatives at stationary points, the type of extremum was determined. The condition for the existence of a maximum (i.e. the value of the determinant higher than zero and the value of the second-order derivatives lower than zero) has been met. After substituting the calculated T and t values into the mathematical models, A^S was determined for the permeabilization processes carried out using ethanol, 1-propanol and 2-propanol (Table 4, 5, 6).

RESULTS AND DISCUSSION

In the presented Tables 4, 5 and 6 it is easy to see the points at which A^S is equal to or close to A^{opt}. This is because the set of determined concentrations also includes optimal or very close concentrations. Significant differences in the estimated A^S confirm the high sensitivity

Table 4. Maximum catalase activities and corresponding temperatures and process times for permeabilization with ethanol

Ethanol concentration (S_E), %	Catalase activity (A_E^S), U·g^{-1}	Temperature (T), °C	Time (t), min
26	650	16.3	48
29	1580	16.1	48
32	2400	16	48
35	3105	15.8	48
38	3700	15.6	48
41	4195	15.5	48
44	4580	15.3	48
47	4850	15.2	48
50	5010	15	48
53	5070	14.8	48
56	5010	14.7	48
59	4850	14.5	48
62	4580	14.3	48
65	4205	14.2	48
68	3700	14	48
71	3100	13.9	48
74	2395	13.7	48

Table 5. Maximum catalase activities and corresponding temperatures and process times for permeabilization with 1-propanol

1-Propanol concentration (S_{1P}). %	Catalase activity (A_{1P}^{S}), U·g^{-1}	Temperature (T), °C	Time (t), min
4	350	12.2	59
6	1080	12.0	57
8	1740	11.8	55
10	2320	11.6	53
12	2830	11.4	51
14	3270	11.3	49
16	3625	11.1	48
18	3910	10.9	46
20	4120	10.7	44
22	4250	10.5	42
24	4310	10.3	40
26	4290	10.2	38
28	4190	10.0	36
30	4030	9.8	35
32	3780	9.6	33
34	3460	9.4	31
36	3070	9.2	29

Table 6. Maximum catalase activities and corresponding temperatures and process times for permeabilization with 2-propanol

2-Propanol concentration (S_{2P}), %	Catalase activity (A_{2P}^{S}), U·g^{-1}	Temperature (T), °C	Time (t), min
8	1970	17.9	62
12	2665	17.6	60
16	3300	17.4	58
20	3870	17.1	56
24	4380	16.9	54
28	4830	16.6	52
32	5220	16.4	50
36	5550	16.1	48
40	5830	15.9	46
44	6040	15.6	44
48	6190	15.4	42
52	6280	15.1	40
56	6300	14.9	38
60	6280	14.6	36
64	6190	14.4	34
68	6045	14.1	32
72	5840	13.9	30

of the permeabilization process to changes in the concentration of the solution used. Similar conclusions have been drawn by Panesar from his studies on the ethanol effectiveness in the permeabilization process of the yeast cells *Kluyveromyces marxianus* [Panesaret al., 2007]. The calculation results also prove the relationship that the higher the concentration of the alcohol solution, the lower the value of the optimal temperature corresponding to A^S.

Table 7. The optimal concentration of alcohol solutions corresponding to A^{opt} and $A^S = ½\ A^{opt}$

Permeabilising agent	Optimal concentration corresponding to A^{opt} , %	Optimal concentration corresponding to $A^S = ½\ A^{opt}$, %
Ethanol	52.6	32.6
1-Propanol	24.2	9.4
2-Propanol	55	15.1

Thus, the financial outlays for obtaining permeabilized yeast cells are related not only to high substrate consumption, but also to cooling costs. For this reason, the effectiveness of the process for fixed, low concentrations of alcohol solutions was analyzed further. According to the data from Tables 4, 5 and 6, lower substrate consumption is associated with a decrease in the effectiveness of the permeabilization process. Therefore, the reference point in the analysis was the concentration of alcohol solutions corresponding to A^S catalase activity, which is half that of A^{opt} (Table 7). The use of an ethanol solution with a concentration 20% lower than the optimal one results in obtaining baker's yeast cells with a catalase activity 50% lower than the optimal A^{opt} activity. Processes using the other two alcohols are more tolerant of reducing the amount of substrate. The concentrations of 1-propanol and 2-propanol solutions for $A^S = ½\ A^{opt}$ are 2.5 times and 3.5 times lower than the optimal concentrations, respectively.

CONCLUSIONS

The presented simulation of increasing the permeability of cell membranes of wet baker's yeast for set concentration values of alcohol solutions allows for easy prediction of intracellular catalase activity. The presented results confirm the sensitivity of the permeabilization process to changes in the concentration of the permeabilization factor. It has been proven that the higher the concentration of the alcohol solution, the lower the optimal temperature value corresponding to the maximum catalase activity. Permeabilization with 2-propanol gives the highest results of permeabilization activity and is the least sensitive to changes in alcohol concentration. The presented simulation allows you to make an informed decision about reducing the consumption of substrates during the permeabilization process and, consequently, also wastes.

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