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Studying and Modeling of the Process Acetification

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Abstract:

Most studies of the process of submerged fermentation consider obtaining spirits vinegar. There are few works related to obtaining wine vinegar. There are no established quantitative dependencies between the intensity of aeration, the temperature of fermentation, the phase of growth, the type of the substrate and the strain of the pure culture acetic acid bacteria etc., and the process parameters. The process of fermentation is best controlled by using modern microprocessor systems provided that there is a validated mathematical model. Studies, concerning the application of mathematical models of the process of acetification for the purpose of controlling and intensifying it, are not available. The influence of the controlling factors remains hidden in the numerical values of the mathematical model coefficients.

This paper studies the influence of the temperature of fermentation and the intensity of aeration on the most important technological and kinetic parameters of the process of acetification. A quantitative estimation of the effect of the temperature of fermentation and the intensity of aeration on the process of fermentation duration, ethanol rate of conversion in acetic acid, lag phase duration and maximum growth rate was made. An appropriate mathematical model has been synthesized for the purposes of controlling and intensifying the process. The Monod's model was used, with the lag phase being taken into account. Dependencies of regression of the basic technological parameters and the kinetic coefficients of the temperature and the intensity of aeration are given.

Keywords — **Wine vinegar, Process acetification, Monod model, Fermentation temperature**

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Abbreviations:

X – biomass concentration [kg/m^3]; S – substrate concentration [kg/m^3]; K_i – constant of inhibition by substrate [kg/m^3]; σ - standard deviation [h]; S – substrate concentration [kg/m^3]; P – product concentration [kg/m^3]; μ - specific rate of growth [h^{-1}]; μ_M - maximum rate of growth [h^{-1}]; K_s - saturation constant [kg/m^3]; K_p - constant of inhibition by product [kg/m^3]; Y – economic coefficient; α - coefficient of conversion of the substrate into product; t_{lp} - duration of the lag phase [h]; P_e is the acetic acid concentration, obtained from the experiment [kg/m^3]; P_M – acetic acid concentration obtained by means of the model [kg/m^3]; S_M – concentration of ethanol, obtained by means of the model [kg/m^3]; X_e – biomass concentration, obtained from the experiment[kg/m^3]; X_M – biomass concentration, obtained by means of the model [kg/m^3]; η is oxidation power (produced acetic acid per 24h) [%]; $Y_{p/x}$ – economic coefficient; Z_1 is a coded variable of the temperature Θ_f;; Z_2 – coded variable of the flow rate Q_{aa}; $S_{initial}$ – initial concentration of ethanol [kg/m^3]; $S_{critical}$ – final concentration of ethanol [kg/m^3].

I. INTRODUCTION

Industrial production of vinegar is carried out practically in every country, which accounts at 8% of the world microbiological industry. On a proposal from FAO, it is accepted, that vinegar is a liquid, containing no less than 4 % acetic acid, produced by means of acetification of agricultural feed-stock. In order to underline its organic origin, this type of vinegar is called fermentation or natural vinegar. In Bulgaria it is typical to manufacture and consume mainly wine vinegar. Appropriate red and white wines are used for the purpose.

There are a number of methods for industrial production of vinegar. In the vinegar industry nowadays, the circulation method is used partially while the submerged fermentation method of acetification is predominant [3,12]. Both in the period of creation and subsequently, most of the research of the submerged fermentation is related to obtaining spirit vinegar. The information about wine substrate, sider and others is limited [4]. The sub-merged fermentation is not fully studied from this point of view. There are no quantitative dependencies between the intensity of aeration, the temperature of fermentation, the phase of growth, the type of the substrate and the pure culture species of acetic acid bacteria etc., and the defining parameters of the process.

The following factors influence the metabolism caused by the acetic acid bacteria and the accumulation of acetic acid: composition of the nutrient medium, temperature of fermentation, aeration of the environment, physiological features of the used species of acetic acid bacteria, pH etc. [1,8,10].

Most effective control of the fermentation process by means of modern microprocessor systems is accomplished when a validated mathematical model of the process is available. There are no studies, reporting application of mathematical models of the process of acetification for the purpose of controlling and intensifying the process itself. The influence of the controlling factors remains hidden in the numerical values of the coefficients of the corresponding mathematical model [5,6,13]. The data, concerning controlling the process of acetification in order to optimize and intensify it, are insufficient. The temperature of fermentation is always constant, and the change in the flow rate of the aerating air is based on acquired manufacturing experience.

Aim of the paper: The paper aims at studying and describing mathematically the process of acetification for the purpose of its control and intensification.

Materials and Methods

All experiments were carried out with red wine, industrial production, suitable for acetification. A pure culture of acetic acid (*A. aceti 100)* bacteria from the Department of Microbiology at University of Food Technologies (UFT) – Plovdiv, Bulgaria was used. The research was carried out in laboratory conditions in the deep semi-continuous working method of Laboratory fermenter Minifer, developed in the Department of Biotechnology at UFT–Plovdiv and automated bioreactor ABR 02M developed by Bulgarian Academy of Sciences (BAS) - Sofia. The temperature of fermentation and the air flow rate are stabilized. For a mathematical description, the kinetics of the process used Monod's models $\mu = \mu_M \frac{S}{K_S + S}$, Monod - Jerusalimskii $\mu = \mu_M \frac{S}{K_S + S}\frac{K_P}{K_P + P}$ and Andrews $\mu = \mu_M \frac{S}{K_S + S + \frac{S^2}{K_i}}$. In determining the values of the technological and kinetic parameters, a computer program based on the least squares method was used.

Results and Discussion

Mathematical model of the process of acetification

Both from the analysis of the process and from literary sources, several models for description of the kinetics of the acetification process were studied, taking into account the lag phase duration. These are: Monod-Jerusalimskii model, taking into account the inhibition by the high concentration of the product; Monod's model and Andrews' model [14,15,17]. For Monod-Jerusalimski model, taking into account the lag phase duration, the system of differential equations has the form [2,16]:

$$\begin{cases} \frac{dX}{dt} = \mu X \\ \frac{dS}{dt} = -\frac{\mu}{Y} X \\ \frac{dP}{dt} = \frac{\alpha\mu}{Y} X \end{cases} \qquad \begin{matrix} \mu = 0 & t \le t_{lp} \\ \mu = \mu_M \frac{S}{K_s + S} \frac{K_p}{K_p + P} & t > t_{lp} \end{matrix} \qquad (1)$$

where:

X – biomass concentration [kg/m^3];

S – substrate concentration [kg/m^3];

P – product concentration [kg/m^3];

μ - specific rate of growth [h^{-1}];

μ_M - maximum rate of growth [h^{-1}];

K_s - saturation constant [kg/m^3];

K_p - constant of inhibition by product [kg/m^3];

Y – economic coefficient;

α - coefficient of conversion of the substrate into product;

t_{lp} - duration of the lag phase [h].

Data have been analyzed about the studied models of acetification process, obtained from experiments in laboratory conditions at values of Θ_f = 28°C and Q_{aa} = 24 dm^3/h, conducted by the submerged fermentation method with pure culture acetic acid bacteria (*A. aceti 100*), taken from the collection of the Department of Microbiology at UFT Plovdiv. The obtained results are shown in Table 1.

Table 1. Kinetic coefficients, defined for the three models

Coefficients Models	μ_M h^{-1}	K_s kg/m^3	t_{lp} h	K_i kg/m^3	K_p kg/m^3	σ h
Monod- Jerusalimskii	0.033	41.45	25.0	-	-66.8	1.39
Monod	0.062	0.709	29.8	-	-	0.347
Andrews	0.15	13.14	24.5	1.2	-	1.49

$\alpha = 0.86$, $Y = 0.02$

where:

K_i – constant of inhibition by substrate [kg/m^3];

σ - standard deviation [h].

Similar results have been obtained from other experiments with red wine substrate at changed temperature Θ_f and low rate Q_{aa}. For all of them the smallest standard deviation - σ (two or three times smaller) is for Monod's model [15]. This gives reasons to assume that Monod's model, taking account of the lag phase, precisely enough describes the process of acetification, conducted by the submerged fermentation method. The constant K_s has very low values in all experiments, within 0.031-2.1 kg/m^3. This illustrates that the specific rate of growth μ has a high value for the whole process, which is close to μ_M. The limitation by substrate is weakly expressed. Fig. 1 presents the curves, both calculated by Monod's model, taking into account the lag phase, and obtained from the experiment at Θ_f = 28°C and Q_{aa}= 24 dm^3/ h.

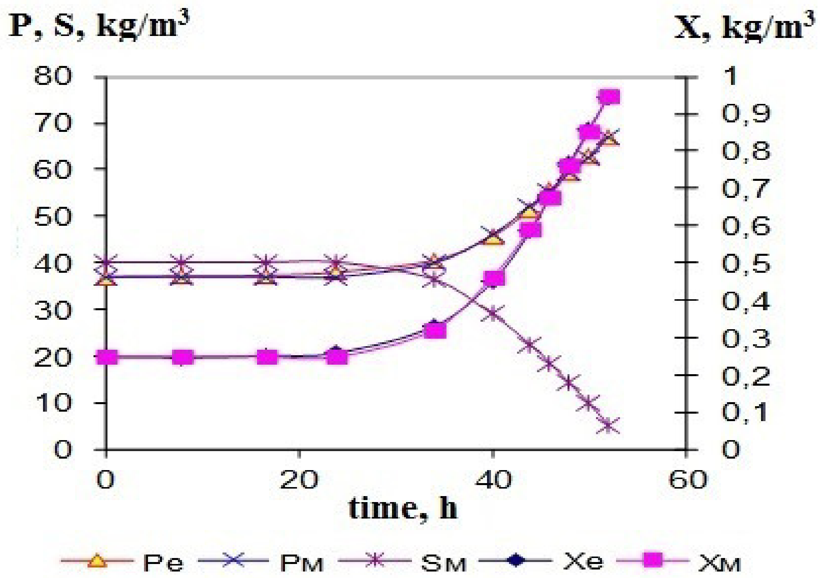

Figure 1. Approximation of the change in P and X by Monod's model, taking into account the lagphase

where: P_e is the acetic acid concentration, obtained from the experiment [kg/m^3];

P_M – acetic acid concentration obtained by means of the model [kg/m^3];

S_M – concentration of ethanol, obtained by means of the model [kg/m^3];

X_e – biomass concentration, obtained from the experiment[kg/m^3];

X_M – biomass concentration, obtained by means of the model [kg/m^3];

The approximation of the experimental data to the chosen model is very good.

Study of the effect of fermentation temperature in submerged acetification

The study aimed at defining the way in which the temperature of the process of acetification influences the basic technological parameters and the kinetic coefficients, defining the process. The exchange rate is 50%. The flow rate is stabilized Q_{aa} = 4 dm^3/h. Five temperature values are set from 24 to 32°C at an interval of 2°C. All experiments are conducted with a pure culture acetic acid bacteria (*A. aceti 100)*, taken from the collection of the Department of Microbiology at the UFT – Plovdiv.

The dependence of certain technological and kinetic coefficients on Θ_f was given in Table 2 [15].

Table 2. Change of basic kinetic and technological coefficients, depending on the temperature of fermentation

Θ_f, °C Coeff.	24	26	28	30	32
η, % for 24 h	2.31	1.882	1.366	2.195	2.006
μ_M, h^{-1}	0.018	0.029	0.042	0.042	0.047
K_s, kg/m^3	0.178	0.298	2.102	1.03	0.887
Y. 10^3	2.64	3.85	6.3	9.46	19.9
$Y_{p/x}$	358.1	215.4	129.3	153.7	70.9

where: η is oxidation power (produced acetic acid per 24h) [%];

$Y_{p/x}$ – economic coefficient.

The oxidation power η is high for all temperatures, while μ_M increases over two times, up to 28°C. The coefficient K_s is small, which confirms that during the process of acetification the limitation by substrate is weakly expressed.

The coefficient $Y_{p/x}$ sharply goes down together with the increase of Θ_f. Consequently, the lower favorable temperatures stimulate the oxidation activity of the acetic acid bacteria A. aceti in submerged cultivation. The change of the important technological parameters (the duration of the process t_p, the lag phase t_{lp}, and the coefficient α) is illustrated in Figure 2.

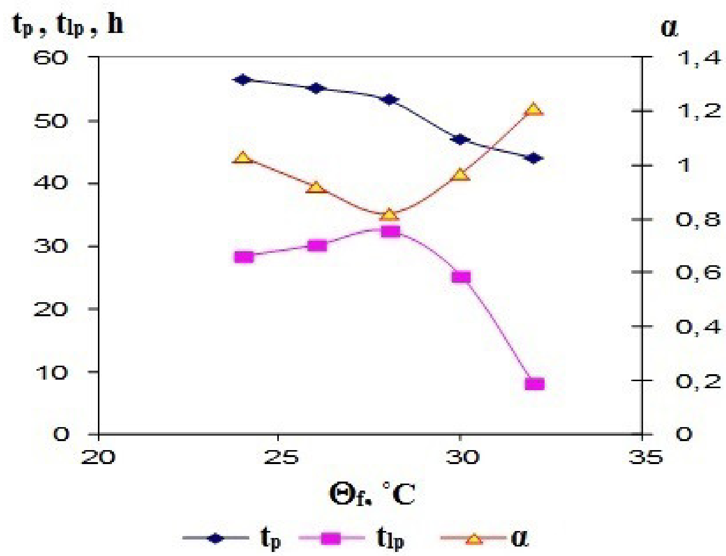

Figure 2. Change of the coefficient α, of the duration of the process t_p, and of the lag phase t_{lp} in dependence on the temperature of fermentation Θ_f

The process of acetification is considered to be completed at $S_{critical}$ = 0,3 vol.%, i.e., $S_{critical}$ = 2,4 kg/m^3. In order to register the duration of the process t_p, the experimental results are extrapolated or interpolated to this value.

With the increase of the temperature, the time t_p constantly decreases, i.e., the process intensifies. For t_{lp} up to 28°C slight increase and then sharp decrease, nearly four times, are observed. Hence, the higher favorable temperatures facilitate the faster adaptation of the acetic acid bacteria to the sharp increase in the concentration of ethanol in the semi-continuous method. The coefficient α has a highest value (α = 1.21) at a temperature Θ_f = 32°C. Therefore, from the point of view of the process intensification, temperatures Θ_f, close to 32°C, are favorable. Cooling will be the least expensive here as well. Similar tests have been carried out for other flow rates – Q_{aa} = 24 and 44 dm^3/h.

Study of the influence of aeration intensity

The influence of aeration intensity on the basic technological and kinetic parameters, defining the process of acetification, has been studied by changing the flow rate of the aerating air Q_{aa}. The experiments were conducted at a temperature Θ_f = 28°C for five different values of the flow rate Q_{aa} : from 4 to 44 dm^3/h at a pace of 10 dm^3/h. Table 3 presents certain important parameters of the process of acetification. For the purpose, the experimental results were processed by a software programme. Monod's model was used, taking into account the lag phase.

Table 3. Basic technological and kinetic parameters, depending on aeration intensity

Q_{aa}, dm^3/h Coefficients	4	14	24	34	44
η,% за 24h	1.366	1.67	1.78	1.477	1.161
μ_M, h^{-1}	0.04	0.048	0.065	0.051	0.039
K_s, kg/m^3	2.102	0.86	0.532	0.467	0.288
$Y.10^3$	6.3	5.9	8.6	7.32	5.19
$Y_{p/x}$	129.3	117.6	94.95	128	114.5

It is obvious that the aeration intensity affects both the growth of acetic acid bacteria and the productivity η. The oxidation power η has a maximum at Q_{aa} = 24 dm^3/h, with nearly 50% increase in comparison to both ends of the interval. The maximum rate of growth μ_M is high for all five values, with an expressed maximum in the middle as well. The increase is by over 50%. The coefficient K_s is low, which proves the weak influence of the limitation by substrate on the process of acetification. The important coefficient $Y_{p/x}$ is high for the whole studied interval, being the lowest at a flow rate Q_{aa} = 24 dm^3/h. Fig. 3 shows the curves of change for the defining technological parameters t_p, α and t_{lp}. The flow rate Q_{aa} influences both the duration of the process t_p and the lag phase weakly, with an expressed minimum in the centre. The change of the coefficient α to this value is insignificant and it sharply goes down for the next two values. This is, probably, due to certain inhibition of oxidation activity of the acetic acid bacteria and mainly to the losses from the high level of aeration.

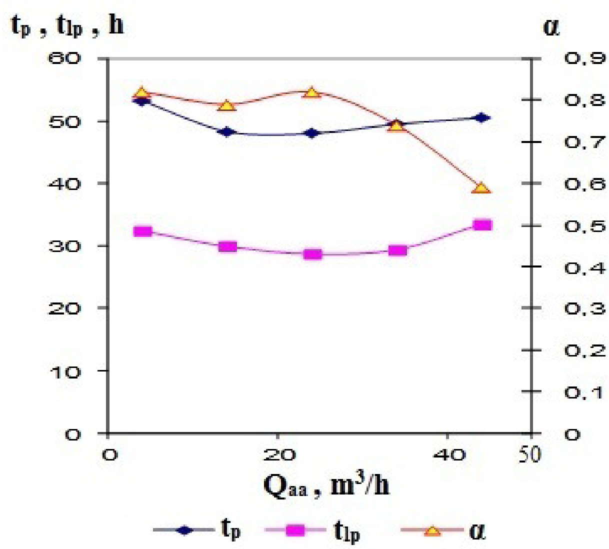

Figure 3. Influence of Q_{aa} on important technological parameters at O_f = 28°C

The influence of the temperature Θ_f is more clearly expressed than the influence of the flow rate Q_{aa}. The most significant is the influence of Q_{aa} on the coefficient α -

decrease after 24 dm^3/h, reaching up to 50%. Consequently, from the point of view of the process intensification, time t_p reduction and minimizing the coefficient α, it is necessary for the flow rate Q_{aa} to be in the range 4÷24 dm^3/h.

Mathematical model of the process of acetification, developed for the purposes of its control and intensification

The preliminary studies of the kinetic of acetification process showed that the process can be described with sufficient for the set of objectives precision by Monod's model, taking into account the lag phase t_{lp}. Most kinetic coefficients have an important and defining role both for controlling and intensifying the acetification process. For example, the quest is that the coefficient α is high, close to the theoretical 1.3, the duration of the lag phase t_{lp} is minimum, which would lead to reduction of the overall duration of the process t_p. The maximum growth rate μ_M should also be high, as the acetic acid is mainly accumulated in young cells in exponential phase of growth.

An important technological parameter, not taken into account by the "global" model, is the duration of the process t_p. The generalized idea [15] is used, and the times t_p and t_{lp} are added to the model as a function of the control factors:

$$\left| \begin{aligned} \frac{dX}{dt} &= \mu X \\ \frac{dS}{dt} &= -\frac{\mu}{Y} X \\ \frac{dP}{dt} &= \frac{\alpha\mu}{Y} X \end{aligned} \right.$$

$$\begin{aligned} &\mu = 0 && t \le t_{lp} \\ &\mu = \mu_M \frac{S}{K_S + S} && t > t_{lp} \end{aligned} \quad . \qquad (2)$$

$$\begin{aligned} \mu_M &= g_1(\Theta_f, Q_{aa}) \\ \alpha &= g_2(\Theta_f, Q_{aa}) \\ t_{lp} &= g_3(\Theta_f, Q_{aa}) \\ t_n &= g_4(\Theta_f, Q_{aa}) \end{aligned}$$

The equation system (2) is a "global" mathematical model of the aceticification, developed for the purposes of its control and intensification. It is necessary to find the functions g1 ÷ g4 and thereby to minimize or maximize the process parameters. An optimal composition plan for two variables is used for the purpose.

The preliminary studies of the influence of Θ_f and Q_{aa} on the basic technological parameters showed that most dependencies are non-linear, have an extremum, and models of second order are appropriate for their mathematical description.

Following the plan of the experimental study, the nine experiments were repeated three times, Substrate of red wine was used in the laboratory installation "Minifer" at the UFT – Plovdiv with pure culture acetic acid bacteria (*A. aceti 100)*. The process variables P and X were measured with two repetitions each, in order to avoid potential subjective errors. The obtained results are shown in a tabular format in a sequence, corresponding to the plan of the experimental study. Table 4 illustrates the averaged results from the first experiment.

Table 4. Experimental data at temperature Θ_f = 24°C and flow rate Q_{aa} = 4 dm^3/h

№1	t,h	0	16	24	32	36	40	44	58
Z_1 = -1	X,kg/m^3	0.21	0.21	0.215	0.228	0.242	0.263	0.281	0.357
Z_2 = -1	P,kg/m^3	35	35.5	36.1	40.9	46.3	54.43	61.4	90.9
	$S_{initial}$ = 54.4 kg/m^3 $S_{critical}$ = 0.07 kg/m^3								

where: Z_1 is a coded variable of the temperature Θ_f;

Z_2 – coded variable of the flow rate Q_{aa};

$S_{initial}$ – initial concentration of ethanol [kg/m^3];

$S_{critical}$ – final concentration of ethanol [kg/m^3].

The obtained results for all points in the experiment plan and for t_p, are shown in Table 5

Table 5. Kinetic coefficients by Monod's model, taking into account t_{lp}

№	Z_1	Z_2	μ_M, h^{-1}	K_s, kg/m^3	t_{lp}, h	Y.10^3	α	t_p, h	σ, h
1	-1	-1	0.018	0.178	28.4	2.64	1.03	56.5	0.27
2	+1	-1	0.047	0.887	8.1	19.9	1.21	44	0.548
3	-1	+1	0.028	1.195	33.9	5.79	0.51	70	0.606
4	+1	+1	0.037	0.473	5.5	13.8	0.84	39.5	0.382
5	-1	0	0.039	0.097	29.9	3	0.52	44	0.328
6	+1	0	0.054	0.631	10.2	16	1.06	37.4	0.107
7	0	-1	0.04	2.102	32.4	6.3	0.82	53.2	0.077
8	0	+1	0.039	0.288	33.5	5.19	0.59	50.5	0.267
9	0	0	0.065	0.532	28.7	8.6	0.82	48	0.371

The analysis of the obtained results from the nine experiments shows that:

a) There is a lag phase and this fact is expressed in the insignificant change of the biomass concentrations X and acetic acid concentration P in the beginning of each process. For the different combinations of factors it varies within a wide range of 5÷10 h to 24÷30 h.

b) The change of the variables, characterizing the process after the lag phase, its duration, the duration of the process t_p, and the final values of P and X considerably depend on the chosen factors and their range of variation.

c) The experiments confirm that it is possible to describe the process of acetification for the purposes of its control and intensification by Monod's model, taking into account t_{lp},

In accordance with the calculated kinetic coefficients for Monod's model, taking into account t_{lp}, the nine processes are built with different combination of factors. The processes, corresponding to the model of S, P and X concentrations, are shown by a continuous line. In order to visually assess the accuracy of approximation by this model, the experimental results for P and X are given as well. Process №1 from Table 4 is shown in Fig. 4.

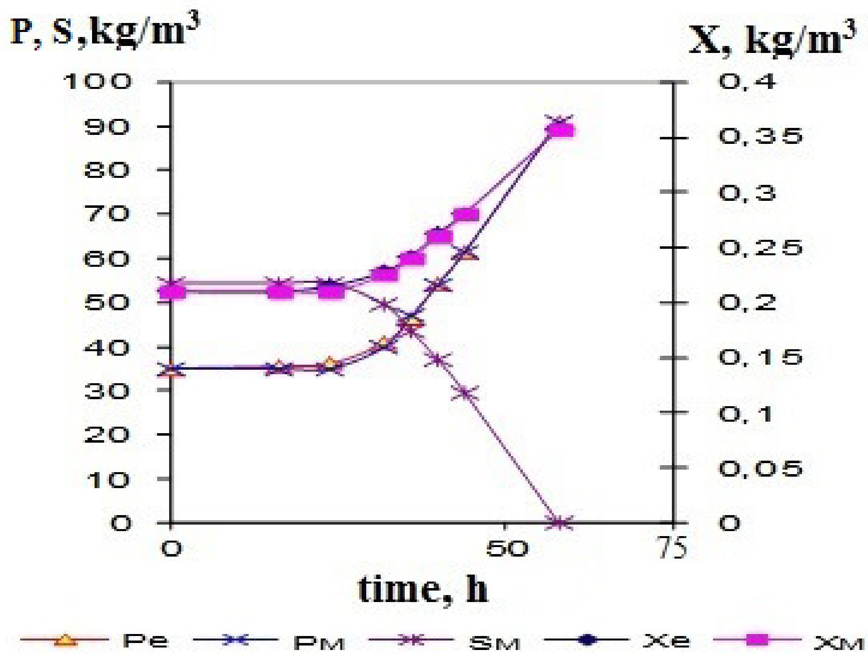

Figure 4. Kinetics of the acetification process at $\Theta_f = 24^\circ C$ and $Q_{aa}=4\ m^3/h$

The analysis of the graphic interpretation of the experimental data (e.g. for fig.4) confirms that under these conditions there is: explicit lag phase, lack of limitation by substrate S and inhibition by product P. The comparison between the experimental data and the calculated values by the model shows precise approximation. Hence, the kinetic of acetification, conducted by the submerged fermentation method, can be described by Monod's model, taking into account t_{lp}. It can be seen from the results, given in Table 5, that both factors - Θ_f and Q_{aa} have considerable influence both on the coefficients and on the time t_p.

In order to build a mathematical model of the process for the purposes of its control and intensification, it is necessary to find the corresponding dependencies g_1-g_4. The results from Table 5 are processed by the methods of regression analysis. After transformations and substitution in the system of equations (2), for a "global" model of the acetification, conducted by the submerged fermentation method, it is obtained:

$$\begin{cases} \dfrac{dX}{dt} = \mu X \\ \dfrac{dS}{dt} = -\dfrac{\mu}{Y} X \\ \dfrac{dP}{dt} = \dfrac{\alpha\mu}{Y} X \end{cases}$$

$$\mu = 0 \qquad t \le t_{lp}$$

$$\mu = \mu_M \frac{S}{K_S + S} \qquad t > t_{lp} \quad . \qquad (3)$$

$$\mu_M = (-551.4 + 38.6\Theta_f + 3.73Q_{aa} - 0.06\Theta_f Q_{aa} - 0.63\Theta_f^2 - 0.05Q_{aa}^2)10^{-3}$$

$$\alpha = 6.28 - 0.43\Theta_f - 0.01Q_{aa} + 0.01\Theta_f^2$$

$$t_{lp} = -522.91 + 41.86\Theta_f + 0.71Q_{aa} - 0.03\Theta_f Q_{aa} - 0.79\Theta_f^2$$

$$t_p = 557.21 - 34.55\Theta_f + 0.58\Theta_f^2$$

$$K_s = 0.709; Y = 9.09.10^{-3}$$

The mathematical model (3) reflects on the relationship between the kinetic coefficients and the basic factors, by means of which the process can be controlled. Adequate regression models of the time t_p and the coefficient α - the two most important parameters of the industrial vinegar production – are obtained. For the purposes of control and intensification regression models of both the lag phase duration t_{lp} and the maximum growth rate μ_M can also be used.

Inadequate models have been obtained for K_s and Y and we suggest that average values be taken for them.

Conclusions

1. It has been established that Monod's model, taking account of the lag phase duration, precisely describes the kinetic of the acetification process, conducted by the submerged fermentation method with different substrates for the purpose of controlling and intensifying it.

2. The influence of the fermentation temperature and aeration intensity on the basic technological microbiological parameters of the acetification process has been confirmed.

3. Quantitative assessment has been made of the influence, which the fermentation temperature in the range 24-36 ° C exerts on the duration of the process of fermentation, on the coefficient α, on the duration of the lag phase and on the maximum growth rate.

4. Considerable influence of the aeration intensity, defined by changing the flow rate of the aerating air, on the defining technological and kinetic parameters has been proved.

5. A "global" mathematical model of the acetification process has been found for the purposes of controlling and intensifying the process. An optimal composition plan has been used for two factors at two levels.

6. Adequate regression dependencies of the basic technological parameters and kinetic coefficients, defining the acetification process, on the temperature and aeration intensity have been defined.

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