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2,3-Butanediol production by *Enterobacter aerogenes*: selection of the optimal conditions and application to food industry residues

P. Perego, A. Converti, A. Del Borghi, P. Canepa

Abstract Optimum values of temperature, pH, and starting substrate concentration are experimentally determined for 2,3-butanediol production by *Enterobacter aerogenes* through three set of batch fermentations of synthetic glucose solutions. The results of tests carried out at variable temperature show an optimum of 39 °C and are used to estimate, for both fermentation and thermal inactivation, the activation enthalpies (7.19 and 23.6 kJ mol^{-1}) and the related entropies (−0.32 and −0.27 kJ mol^{-1} K^{-1}). An optimum pH value of 6.0 is evidenced from batch runs at variable pH, whose results are also used to make reasonable hypotheses on the reaction controlling the metabolic pathway which leads to butanediol. The fermentability of different food industry wastes, namely starch hydrolysate, both raw and decoloured molasses, and whey, is finally checked.

List of symbols

A	h^{-1}	pre-exponential factor of fermentation
B	h^{-1}	pre-exponential factor of thermal inactivation
K_a		acid ionisation constant
K_b		basic ionisation constant
K_R		ionisation constant of a generic aminoacidic R group
P	$g_P\ l^{-1}$	product (butanediol) concentration
R	kJ mol^{-1} K^{-1}	ideal gas constant
S	$g_S\ l^{-1}$	substrate concentration
t	h or days	fermentation time
T	°C or K	temperature
X	$g_X\ l^{-1}$	cell mass concentration
Y	$mol_P\ mol_S^{-1}$	product yield on starting substrate
$Y_{P/X}$	$g_P\ g_X^{-1}$	product yield on biomass

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Greek letters

Δh^*	kJ mol^{-1}	activation enthalpy of fermentation
$\Delta h^{*\prime}$	kJ mol^{-1}	thermodynamic quantity defined in Eq. (3)
Δh_d^*	kJ mol^{-1}	activation enthalpy of thermal inactivation
Δs^*	kJ mol^{-1} K^{-1}	activation entropy of fermentation
Δs_d^*	kJ mol^{-1} K^{-1}	activation entropy of thermal inactivation
v	$mmol_P\ l^{-1}\ h^{-1}$	volumetric productivity
v_s	h^{-1}	specific productivity

Subscripts

f	value at the end of fermentation
m	average value calculated at the end of fermentation
0	starting value
opt	optimum value
P	product
S	substrate
X	biomass

1 Introduction

2,3-Butanediol is a very important chemical because of its wide commercial applications in the manufactures of butadiene [1] and butan-2-one [2], aviation fuels, printing inks, perfumes, fumigants, moistening agents, explosives, and plasticizers, as well as of its use as antifreeze agent, liquid fuel [2–4], and octane booster [5]. In addition to these applications, butanediol can be used as carrier for pharmaceuticals and raw material for the production of diacetyl (a flavouring agent), for which the biotechnological production (fermentation) appears to be more friendly and economically more interesting than the traditional chemical methods [6].

2,3-Butanediol can be produced from both hexoses and pentoses by several microorganisms, among which *Klebsiella oxytoca*, *Bacillus polymyxa*, *Bacillus licheniformis*, *Bacillus subtilis*, *Aeromonas hydrophila*, *Serratia marcescens* [3, 7], and *Bacillus amyloliquefaciens* [5]. The factors influencing butanediol production can be either nutritional factors, like substrate and product concentrations as well as medium supplements, or cultural factors, like temperature, pH, aeration, and inoculum [7].

Enterobacter aerogenes, a facultative anaerobic bacterium, produces preferentially hydrogen under strictly anaerobic conditions [8] and turns into a butanediol producer very similar to *Klebsiella oxytoca* when fermentation is stimulated by microaeration [9–13].

Although the aeration conditions for this fermentation have been deeply studied by Zeng et al. in terms of respiratory quotient and oxygen transfer rate [9–13], a complete optimisation study of the remaining operating parameters, like those available for other butanediol producers [3, 14], could be the key of future improvements of both product yield and productivity. The necessity of these advances is demonstrated by the fact that low product concentrations in the broth, besides the additional problems of high boiling point and hydroscopicity [3, 4], implies high recovery costs.

In addition, a certain number of natural substrates, like waste sulphite liquor, food industry wastes, molasses, sugar beet pulp, wood hydrolysates, and agricultural wastes, have been successfully fermented by *Aeromonas hydrophila*, *Bacillus polymyxa* and *Klebsiella oxytoca* [3], but no similar study is reported for *E. aerogenes*.

Aim of this work is to optimise the operating conditions of 2,3-butanediol production from synthetic glucose solutions by *E. aerogenes* (except for aeration) and to apply these results to the conversion of food industry wastes.

2 Materials and methods

2.1 Microorganism

Pure cultures of *E. aerogenes* (NCIMB 10102) used in this study were maintained on nutrient agar slants at 4 °C and subcultured monthly. The cells were then incubated aerobically at 37 °C in shaken flasks on a rotary shaker at 150 rpm and harvested at the end of the exponential phase.

The pre-culture medium was nutrient broth containing 2.0 g l^{-1} yeast extract, 5.0 g l^{-1} peptone, 5.0 g l^{-1} NaCl, and 1.0 g l^{-1} beef extract, sterilised at 120 °C for 15 min, and supplemented with the carbon source at the same concentrations as those used in the fermentations. The cells were grown for 48 h, centrifuged, and aseptically inoculated into the fermentor.

2.2 Media

Preliminary optimisation tests were carried out on a synthetic medium containing 5.0 g l^{-1} yeast extract, 6.0 g l^{-1} KH_2PO_4, 14.0 g l^{-1} K_2HPO_4, 2.0 g l^{-1} $(NH_4)_2SO_4$, 1.0 g l^{-1} sodium citrate dihydrate, and 0.2 g l^{-1} $MgSO_4 \cdot 7H_2O$, supplemented with glucose up to the desired starting concentration.

Further tests were performed utilising food industry by-products as substrates, namely starch hydrolysate from corn transformation, molasses from sugar extraction from beet, and whey from cheese manufacture, whose compositions are listed in Table 1. Corn starch hydrolysate, supplied by Roquette Italia SpA, Cassano Spinola (Italy), was diluted with tap water up to a glucose concentration of 20 g l^{-1} and by adding the following salts: 6.0 g l^{-1} KH_2PO_4, 14.0 g l^{-1} K_2HPO_4, 2.0 g l^{-1} $(NH_4)_2SO_4$, and 0.4 g l^{-1} $MgSO_4 \cdot 7H_2O$. Molasses, supplied by Eridania SpA, Genova (Italy), was previously diluted with tap water up to 20 g l^{-1} sucrose and then fermented either directly or after treatment up to 50% of the original colour in a column filled with a Amberlite IRA900 ionic-exchange resin to remove high molecular weight inhibitors. Prior to fermentation, whey, supplied by Fratelli Pinna Industria Casearia, Sassari (Italy), was ultrafiltrated and treated for 30 min at 30 °C with 10 ml l^{-1} of an industrial preparation of β-galactosidase, Lactozyme 3000L HP-G of Novo Nordisk SpA, Milan, which ensured a hydrolysis conversion higher than 75%. Sodium citrate dihydrate and NaOH were used to buffer the media at pH 6.0.

Table 1. Composition of the food industry by-products utilised as substrates

Component	Amount
(a) Starch hydrolysate	
Dry extract	69.5%
Glucose	85.0% of dry extract
Maltose	2.6% of dry extract
Trisaccharides	0.7% of dry extract
Oligosaccharides	6.85% of dry extract
Ashes	3.8% of dry extract
Proteins (N 6.25)	1.05% of dry extract
Ca^{2+}	0.305 mg g^{-1}
Mg^{2+}	0.129 mg g^{-1}
Na^{+}	71.5 mg g^{-1}
K^{+}	1.09 mg g^{-1}
(b) Molasses	
Sucrose	495 mg g^{-1}
Ca^{2+}	8.40 mg g^{-1}
Mg^{2+}	9.87 mg g^{-1}
Na^{+}	24.3 mg g^{-1}
K^{+}	34.3 mg g^{-1}
(c) Whey	
Dry extract	5.85%
Lactose	4.0% of dry extract
Ashes	0.42% of dry extract
Proteins (N 6.25)	0.55% of dry extract
Not proteic N	0.48% of dry weight
Lipids	Absent

2.3 Operative conditions

A 2.0 l Gallenkamp FBL-195 bioreactor with a working volume of 1.5 l, stirred at 150 rpm, was employed for batch fermentations.

The pH of the fermentation broth was automatically controlled, with an accuracy of 0.1 pH units, by a pH control module FBL-725 provided with a peristaltic pump, which injected a fine stream of 30% NaOH solution. The free access of air into the reactor was allowed by means of cotton caps and no control of the dissolved oxygen was operated. The temperature was kept at the selected value by a temperature control module FBL-360. The fermentor as well as the media were sterilised by autoclaving at 120 °C for 15 min.

2.4 Analytical methods

Butanediol and acetoin concentrations were determined by gas chromatography using Cromosorb 101 operated with N_2 as the carrier gas, at 250 °C evaporation temperature,

300 °C detector temperature, and 175 °C column temperature, and using *n*-butanol as the internal standard. Butanediol and acetoin were finally expressed as butanediol for simplicity.

Cell mass concentration was determined by filtering a known volume of culture broth through 0.2 μm autoclavable filters. The filters were dried at 105 °C, until no weight change between consecutive measurements was observed. All the fermentations were carried out with an inoculum of 0.25 $g_X\ l^{-1}$ (dry weight). A few millilitres of a thick suspension of the microorganisms, obtained by aseptic centrifugation, whose biomass concentration was previously determined as reported above, was added to the medium until the desired biomass level was attained.

The concentrations of sugars in the broth as well as their contents in the natural substrates were determined by means of a HPLC Hewlett Packard 1100 using IR-detector. A column Hypersil 200 × 4.6 mm was used at 35 °C with 80:20 acetonitrile/water as the mobile phase at a flow rate of 1.0 ml min^{-1}.

Raw proteins were determined by the Kjeldahl method [15] and metallic ion concentrations by a Perkin-Elmer 5000 Atomic Absorption Spectrophotometer [16].

3
Results and discussion

In order to improve the production of 2,3-butanediol by *E. aerogenes*, an optimisation study, devoted to the selection of the optimal values of temperature, pH, and starting substrate concentration, has been carried out. An additional set of fermentation runs has been done at the end of this study using different food industry wastes as raw materials, namely starch hydrolysate, raw and decoloured molasses, and whey.

3.1
Effect of temperature

The effect of temperature on this fermentation process has been investigated carrying out seven batch tests at temperatures varying from 23 up to 46 °C and keeping constant the rest of the operating conditions, whose results have been used for the first time to our knowledge, to estimate the thermodynamic quantities of this process.

In order to exclude any possible inhibition due to the presence of inhibitors in the raw material as well as to the occurrence of a substrate saturation phenomenon, synthetic glucose solutions with a very low starting substrate level (20 $g_S\ l^{-1}$) have been preferred. A relatively low starting biomass level has also been selected (0.25 $g_X\ l^{-1}$) to make possible an accurate calculation of both volumetric and specific productivities, which are the kinetic parameters more commonly used for estimating the thermodynamic quantities of both the fermentation process and the thermal inactivation of the biocatalyst. The pH has been kept at 5.5, which is the value more frequently reported in the literature for the production of 2,3-butanediol by this micro organism [12, 13] while starting microaerophilic conditions without any control of dissolved oxygen were selected for simplicity.

The main results of these tests listed in Table 2 show that the yield of glucose conversion into 2,3-butanediol and acetoin considered as a whole (Y) as well as the final product concentration in the fermented broth (P_f) are both decreasing functions of temperature within the range of temperature tested. In particular, these parameters seem to slightly decrease with increasing temperature up to 39 °C, beyond which a sharp fall takes place because of the thermal inactivation of the enzymes at high temperature.

Less significant is, on the other hand, the influence of temperature on the fermentation time, which progressively decreases from 12.5 days up to 10.4 days with increasing the temperature from 23 to 46 °C. This effect, which is a consequence of the process of acceleration due to a temperature increase is completely counterbalanced, at high temperature, by the thermal inactivation. The combination of these opposite phenomena is responsible for a constancy of the fermentation time over 39 °C.

A different behaviour can be observed for the 2,3-butanediol productivity, which progressively increases with the temperature up to 39 °C and decreases over this threshold. Figure 1 shows this peculiar thermal effect on the average productivity, v_m which is reported, according to Arrhenius, in a semilogarithmic plot versus the reciprocal temperature. Below 39 °C (right side of this figure), the logarithm of productivity linearly decreases with increasing $1/T$, whereas, over that threshold (left side), it goes in the opposite direction.

In previous studies dealing with other fermentation or enzymatic processes, the reasons of this peculiar behaviour of biosystems, with respect to traditional chemical system, has clearly been explained [17–19]. The formation of the activated state is likely to be coupled with the

Table 2. Effect of temperature on 2,3-butanediol production by *E. aerogenes*. S_0 = 20 $g_S\ l^{-1}$; X_0 = 0.25 $g_X\ l^{-1}$; pH = 5.5

T (°C)	P_f ($g_P\ l^{-1}$)	t_f (d)	Y ($mol_P\ mol_S^{-1}$)	v_0 ($mmol_P\ l^{-1}\ h^{-1}$)	v_m ($mmol_P\ l^{-1}\ h^{-1}$)
23	8.3	12.5	0.83	0.90	0.31
28	8.1	11.7	0.81	1.12	0.32
33	8.0	11.0	0.80	1.35	0.34
39	8.0	10.4	0.80	1.59	0.36
41	7.8	10.4	0.78	1.38	0.35
43	7.5	10.5	0.75	1.02	0.33
46	7.0	10.4	0.70	0.51	0.31

P_f = final product concentration
t_f = total fermentation time
Y = yield of butanediol on starting substrate
v_0 = starting volumetric productivity
v_m = average volumetric productivity calculated at the end of fermentation

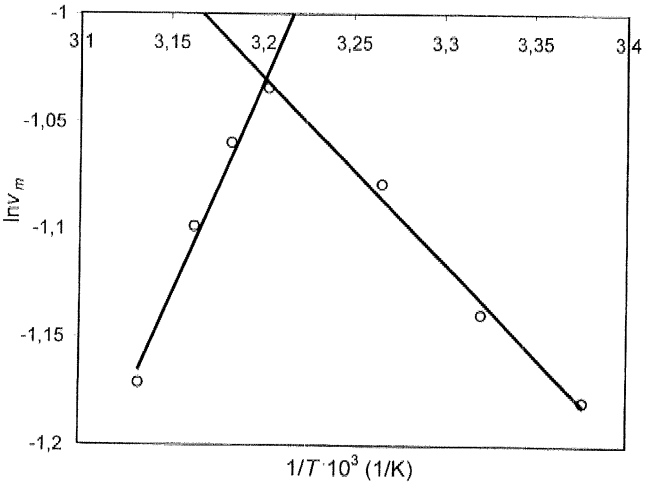

Fig. 1. Arrhenius plot for the estimation of the thermodynamic quantities of both 2,3-butanediol fermentation by *E. aerogenes* and thermal deactivation

phenomenon of thermal inactivation of the biocatalyst (both microbial and enzymatic systems, which kinetically acts in the opposite direction and, whose relative significance grows with increasing temperature. As a consequence, below a temperature threshold (T_{opt} = 39 °C in the present case), the positive kinetic effect of a temperature increase on the activated state formation prevails over the negative effect on the biocatalyst activity and stability, thus following an Arrhenius-type behaviour:

$$\ln \nu_m = \ln(AXY_{P/X}) - \Delta h^*/RT \quad T < T_{opt} \ , \qquad (1)$$

where A is the Arrhenius pre-exponential factor linked to the activation entropy of the fermentation process, Δh^* is the activation enthalpy of the fermentation process, R the ideal gas constant, T the absolute temperature, X is the biomass concentration, and $Y_{P/X}$ the yield of product on biomass.

At temperature higher than the optimum, on the contrary, an opposite behaviour is observed, As previously explained in detail [18], this linear decrease of productivity can also be described an Arrhenius-type equation:

$$\ln \nu_m = \ln(BXY_{P/X}) - \Delta h^{*'}/RT \quad T > T_{opt} \ , \qquad (2)$$

where:

$$\Delta h_d^* = \Delta h^* + |\Delta h^{*'}| \ , \qquad (3)$$

and B are the activation enthalpy and a sort of pre-exponential factor of thermal inactivation, respectively.

The activation enthalpies (Δh^* and Δh_d^*) and the pre-exponential factors (A and B) of both fermentation and thermal deactivation have been estimated in this study from the slopes and the intercepts on the ordinate axis of the straight lines, while the related activation entropies Δs^* and Δs_d^*) have been estimated from the values of A and B by standard methods [17–19].

According to this approach, the values listed in Table 3 have been estimated for the main thermodynamic quantities from the plots of Fig. 1. While the activation entropies for both 2,3-butanediol fermentation and thermal deactivation are comparable with those estimated for

Table 3. Thermodynamic quantities estimated with the Arrhenius approach for 2,3-butanediol fermentation from glucose by *E. aerogenes*

	Fermentation	Thermal deactivation
Activation enthalpy (kJ mol^{-1})	7.19	23.6
Activation entropy (kJ mol^{-1} K^{-1})	−0.32	−0.27
r^2	0.992	0.981

r^2 = determination coefficients referred to the straight lines described by Eqs. (1) and (2)

alcohol fermentation by *Saccharomyces cerevisiae* (Δs^* = −0.20 kJ mol^{-1} K^{-1} and Δs_d^* = −0.21 kJ mol^{-1} K^{-1}) [18], the related activation enthalpies are quite lower (Δh^* = 40 kJ mol^{-1} and Δh_d^* = 80 kJ mol^{-1} for alcohol fermentation) which means that this fermentation, system is particularly sensitive to temperature if compared with other bioprocesses, even if the macroscopic effects of this sensitivity seem to appear at higher temperatures. Nevertheless, a collection of the activation enthalpy values reported in the literature for quite different systems (enzymatic catalysis, cell growth, fermentations, etc.) [17–19] shows that these are always of the same order of magnitude (54–71 kJ mol^{-1} for microbial growth, 60–100 kJ mol^{-1} for enzyme denaturation, and 57–105 kJ mol^{-1} for fermentations) [18, 20–22], the only exception being the thermal microbial death, which is characterised by a much higher activation enthalpy (290–380 kJ mol^{-1}) [20].

This comparability among thermodynamic quantities of completely different bioprocesses suggests not only that different enzymatic systems are probably subject to similar deactivation mechanisms, but also that the thermal activation of microbial systems could have an enzymatic origin. In other words, the thermal inactivation of whole cell systems is probably associated to the progressive denaturation of the enzyme, which kinetically controls a fundamental metabolic pathway. More information on the nature of this enzyme or this pathway in *E. aerogenes* could be got, in addition to the traditional metabolic studies, from the analysis of the pH effect on productivity, a preliminary attempt of which is shown in the following section.

Since one of the aims of this work is to accelerate 2,3-butanediol fermentation, the temperature (39 °C) which ensured the highest productivity (0.36 mmol$_P$ l^{-1} h^{-1}) has been selected for the successive tests of optimisation of the remaining operating conditions.

3.2 Effect of pH

As well known, the control of pH in bioprocesses, which lead to several products is particularly important because this parameter can influence the bacterial metabolism [23]. To study the influence of pH on the 2,3-butanediol fermentation by *E. aerogenes*, an additional set of batch tests has been performed at variable pH and keeping constant the other operating conditions, namely T = 39 °C, S_0 = 20 g$_S$ l^{-1}, and X_0 = 0.25 g$_X$ l^{-1}.

From the main results of these tests, which are summarised in Table 4, it can be seen that the pH dependence of the conversion yield is quite different from the temperature dependence. In fact, while this parameter keeps nearly constant within a narrow range of pH ($5.5 < pH < 6.5$), it sharply decreases either at lower or at higher pH values, with the stronger effect being detected under acid conditions. This means that the metabolic pathway, which leads to 2,3-butanediol formation is even more sensitive to pH variation than to temperature variation. It is possible that, under extreme conditions, the activity of one of more of the key enzymes of this route is reduced to such an extent that other alternatives become preferable to get energy.

As explained by Gard and Jain [7], as a general rule, alkaline conditions (above pH 6.3–6.5) favour the formation of organic acids, with a corresponding decrease in the yield of butanediol. On the other hand, excess acid conditions usually inhibit the biomass growth as well as the bioprocess itself. An optimum range for 2,3-butanediol production between 5.5 and 6.5 could then be the optimal combination of these effects.

An unexpected behaviour of the total fermentation time is also evident from the data of Table 4. The observation that shorter times correspond just to the lower conversion yields can be justified supposing that the main alternative metabolic route (acids production) is, under alkaline condition, quicker than the 2,3-butanediol fermentation, thus reducing the amount of carbon source available for this route and then the time necessary to consume it. On the other hand under excess acid conditions, the fermentation rapidly stops because of biomass inhibition. These results on the whole indicate an optimum pH of 6.0, which is close enough to the value that Zeng et al. considered optimal for this production [12, 13].

In order to shed light on the nature of the pH influence on productivity, the graphic technique of Villadsen et al. [24] has been employed. This technique, which has recently been used with success for xylitol production from detoxified hemicellulose hydrolysates [25], is based on the hypothesis that the active form of a generic enzyme-substrate complex is in equilibrium with other two alternative (inactive) forms, which result from an acid ionisation (at pH lower than optimum) and a basic ionisation (at pH higher than optimum), respectively. A pH variation in the reaction environment and/or the active site can lead to a modification of the ionisation state of aminoacidic R groups and then to the deactivation of a fraction of the controlling enzyme, with consequent decrease of the specific productivity (ν_{sm}), according to the equation:

$$\nu_{sm} = \nu_{sm,opt}/(1 + [H^+]/K_a + K_b/[H^+]) \quad , \qquad (4)$$

where $\nu_{sm,opt}$ is the specific productivity under optimal pH conditions for 2,3-butanediol production, while K_a and K_b are the acid and basic ionisation constants of the two main ionisable groups that are more likely involved in the catalysis by a key enzyme controlling this metabolic pathway.

Since the contribution of the term $[H^+]/K_a$ at the denominator of Eq. (4) becomes predominant at low pH, neglecting the other terms, we obtain, at every point lying on the horizontal line of specific productivity, $\nu_{sm} = \nu_{sm,opt}$ and then pK_a = pH. This allows one to estimate the value of pK_a from the intercept of the oblique straight line on the left (referring to $pH < pH_{opt}$), with that parallel to the abscissae axis (referring to $pH = pH_{opt}$). Analogously, pK_b can be calculated.

In the present case, however, the values of pK_a and pK_b that can be estimated for *E. aerogenes* from the straight lines of Fig. 2 are both very close to 6.0 (horizontal line practically absent), that is the optimum pH experimentally obtained in this work for 2,3-butanediol production. This value could correspond to the imidazole ionisable R group of a histidin residue, which notoriously exhibits an intermediate acid-basic behaviour ($pK_R = 6.0$) [26], or , more likely, to the tiazolic ring of the tiamine pyrophosphate, which is employed as prostetic group by the acetyl lactate synthase. This enzyme, or the acetyl lactate decarboxylase, which catalyses the subsequent decarboxylation of acetyl lactate to acetoin [3], could then be the one more likely to control this fermentation.

Table 4. Effect of pH on 2,3-butanediol production by *E. aerogenes*. $S_0 = 20\ g_S\ l^{-1}$; $X_0 = 0.25\ g_X\ l^{-1}$; $T = 39\ °C$

pH	P_f ($g_P\ l^{-1}$)	t_f (d)	Y ($mol_P\ mol_S^{-1}$)	ν_m ($mmol_P\ l^{-1}\ h^{-1}$)	ν_{sm} (h^{-1})
4.0	2.5	5.8	0.25	0.20	0.036
4.5	4.6	9.2	0.46	0.23	0.042
5.0	7.7	12.5	0.77	0.28	0.051
5.5	7.8	11.5	0.78	0.32	0.057
6.0	8.0	10.4	0.80	0.36	0.064
6.5	7.2	10.0	0.72	0.33	0.060
7.0	6.3	9.6	0.63	0.30	0.055
8.0	5.2	9.6	0.52	0.25	0.045

P_f = final product concentration
t_f = total fermentation time
Y = yield of butanediol on starting substrate
ν_m = average volumetric productivity calculated at the end of fermentation
ν_{sm} = average specific productivity calculated at the end of fermentation

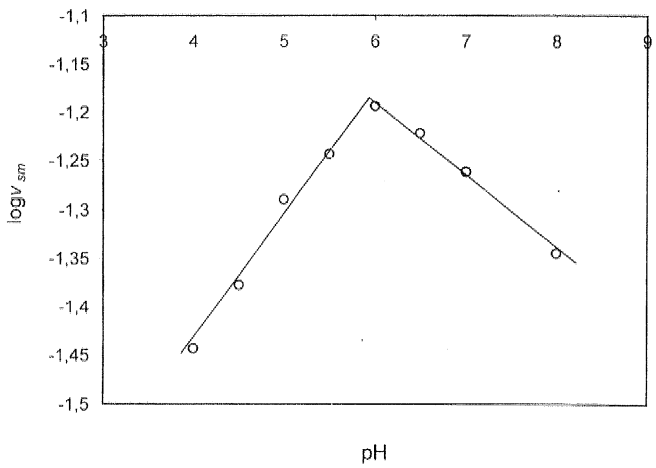

Fig. 2. Estimation of pK_a and pK_b of ionisable functional groups of the enzyme likely controlling butanediol production by *E. aerogenes*

Table 5. Effect of starting glucose concentration on 2,3-butanediol production by *E. aerogenes*. pH = 6.0; X_0 = 0.25 g l^{-1}; T = 39 °C

S_0 (g_S l^{-1})	P_f (g l^{-1})	t_f (d)	Y (mol_P mol_S^{-1})	v_0 ($mmol_P$ l^{-1} h^{-1})	v_m ($mmol_P$ l^{-1} h^{-1})
20	8.0	10.4	0.80	0.32	0.36
35	13.9	16.0	0.74	0.37	0.40
50	18.0	20.8	0.72	0.52	0.40
75	25.5	24.4	0.68	0.47	0.48
100	33.0	29.2	0.66	0.42	0.52

P_f = final product concentration
t_f = total fermentation time
Y = yield of butanediol on starting substrate
v_0 = starting volumetric productivity
v_m = average volumetric productivity calculated at the end of fermentation

3.3
Effect of starting glucose concentration

Further tests at different starting glucose concentrations have been carried out in order to put in evidence possible diffusion limitations and/or inhibition due to excess substrate on 2,3-butanediol fermentation. The main results of these tests, which have been performed varying S_0 from 20 to 100 g_S l^{-1} and keeping constant the other operating parameters (pH = 6.0; T = 39 °C), are listed in Table 5. These results show that the fermentation time gradually grows and the conversion yield lowers with increasing the starting substrate level, which is in agreement with what observed for most fermentation processes.

As far as the starting volumetric productivity is concerned, Fig. 3 shows that this parameter progressively increases with S_0 up to a maximum value, over which it decreases. This behaviour demonstrates the existence of a S_0 threshold (ca. 50 g_S l^{-1}) below which the substrate diffusion from the bulk to the cell inside is probably the phenomenon controlling the 2,3-butanediol fermentation; over this threshold, on the contrary, the process is reaction-controlled. The decrease in productivity observed for $S_0 \geq 50$ g_S l^{-1} can be ascribed, on the other hand, to the inhibition due to excess substrate.

This behaviour is absent in other fermentation processes, like alcohol fermentation, for which Ciftci et al. demonstrated an exponential decrease of v_0 with S_0 [27]. A similar effect, on the other hand, has been reported for other biological systems by several authors, who ascribed it to the significance of maintenance contribution at low S_0 levels [28]. In our case, however, this phenomenon should reasonably be excluded considering the progressive decrease of the conversion yield detected with increasing S_0.

The productivity values obtained in this study under optimised conditions of temperature, pH, and starting substrate concentration appear to be much lower than those reported by Zeng et al. at higher biomass levels and with oxygen transfer rate and respiratory quotient control [13]. This should advise us to include also these additional environmental and cultural factors in a wider optimisation attempt.

3.4
Fermentation of food industry wastes

The potential of this fermentation process from the industrial point of view can be checked by utilising sugar residues as raw materials. We have selected food industry wastes in this study because of their high sugar content and their compatibility with biological processes, namely starch hydrolysate coming from corn transformation, both raw and decoloured molasses coming from sugar extraction from beet, and whey from cheese manufacture. The experimental data collected from batch fermentations done at the same pH (6.0), temperature (39 °C), and starting substrate concentration (20 g_S l^{-1}) (Fig. 4), have

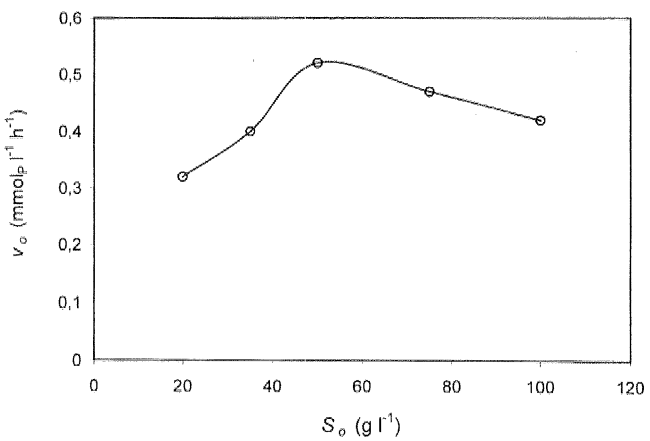

Fig. 3. Dependence of the starting butanediol productivity on the starting glucose concentration

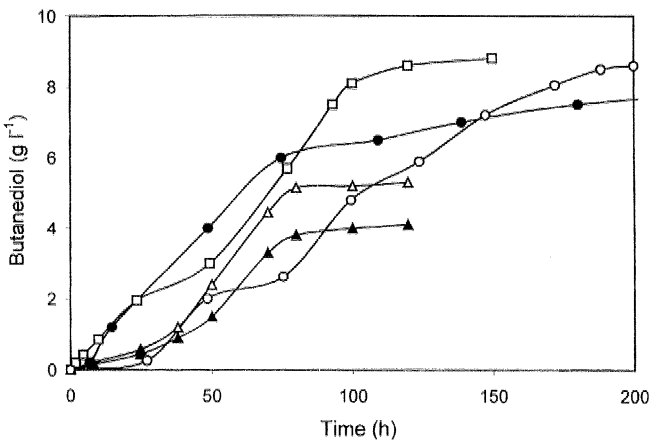

Fig. 4. 2,3-Butanediol production by *E. aerogenes* from different substrates. T = 39 °C; pH = 6.0; S_0 = 20 g_S l^{-1}. (●) Glucose; (□) Starch hydrolysate; (△) Decoloured molasses; (▲) Raw molasses; (○) Whey

Table 6. 2,3-Butanediol fermentation of different food industry wastes by *E. aerogenes*. pH = 6.0; X_0 = 0.25 $g_X\ l^{-1}$; T = 39 °C; S_0 = 20 $g_S\ l^{-1}$

Substrate	P_f ($g_P\ l^{-1}$)	t_f (d)	Y ($mol_P\ mol_S^{-1}$)	v_m ($mmol_P\ l^{-1}\ h^{-1}$)
Glucose	8.0	10.4	0.80	0.36
Starch hydrolysate	8.8	6.2	0.88	0.65
Raw molasses	4.1	5.0	0.41	0.38
Decoloured molasses	5.3	5.0	0.53	0.49
Whey	8.6	8.3	0.86	0.48

P_f = final product concentration
t_f = total fermentation time
Y = yield of butanediol on starting substrate
v_m = average volumetric productivity calculated at the end of fermentation

been utilised to calculate the butanediol yield and productivity for each one of these substrates (Table 6).

It should be noticed that starch hydrolysate is by far the most interesting raw material, ensuring the highest product yield ($Y = 0.88\ mol_P\ mol_S^{-1}$) and volumetric productivity ($v_m = 0.65\ mmol_P\ l^{-1}\ h^{-1}$). A productivity, which is nearly twice as estimated for synthetic glucose solutions, confirms the existence in this material of growth factors which are able to stimulate microbial metabolism [29, 30].

Molasses are consumed more quickly with respect to the other substrates, but the product yield is always unsatisfactory ($Y = 0.38\ mol_P\ mol_S^{-1}$). Even the decolourisation with ionic-exchange resins to remove polymeric inhibitors [31] was not able to improve the fermentability of this material at acceptable levels, ensuring only increases of about 30% in both the yield and productivity.

The most interesting results, however, appear to be excellent fermentation data obtained with pre-hydrolysed whey, a food waste of difficult disposal in Italy, whose territory is completely dominated by cheese manufacture. This material was fermented at a rate of $0.48\ mmol_P\ l^{-1}\ h^{-1}$, which is comparable to the test of decoloured molasses, but ensured a final product yield ($Y = 0.86\ mol_P\ mol_S^{-1}$) very close to that shown by starch hydrolysate.

4 Conclusions

This study dealt with the optimisation of the operating conditions for 2,3-butanediol production by *E. aerogenes* from synthetic glucose solutions as well as from food industry wastes.

Tests carried out at variable temperature demonstrated that the best temperature for this system is 39 °C and allowed us to estimate the main thermodynamic quantities of both fermentation and thermal inactivation.

A second set of batch runs at variable pH evidenced an optimum value for this parameter around 6.0, which could be considered as the best compromise between the organic acids overproduction under alkaline conditions and the microbial metabolism inhibition under acid conditions.

Finally, among the different food industry wastes tested in this study, starch hydrolysate coming from corn transformation and whey from cheese manufacture exhibited the most interesting results in terms of both product yield and productivity.

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