# THE INFLUENCE OF THE INITIAL CONCENTRATIONS OF GLUCOSE AND YEAST EXTRACT ON THE ETHANOLIC FERMENTATION BY *Pachysolen tannophilus*

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The ethanolic fermentation in batch cultures of *Pachysolen tannophilus* was studied experimentally varying the initial concentrations of two of the components in the culture medium: glucose between 0 and 200 g $l^{-1}$ and yeast extract between 0 and 8 g $l^{-1}$. The yeast extract appears to be a significant component both in cell growth and for ethanol production.

The conversion of lignocellulosic residues to ethanol undergoes an initial hydrolysis which is usually performed with diluted acids (acid hydrolysis). During the process pentose and hexose monosaccharides are released to be later converted to ethanol by appropriate microorganisms. The yeasts used traditionally, of the *Saccharomyces* strain, only produce ethanol from hexoses and not from pentoses as they would not benefit wholly from the hydrolyzed solutions obtained from lignocellulosic residues. In this sense, one of the most interesting yeasts is *Pachysolen tannophilus* due to its capacity to ferment both hexoses and pentoses, basically glucose and xylose which are the most abundant components after hydrolysis of the residual biomass. Its capacity to ferment monosaccharides D-manose and D-galactose[1] and even D-cellobiose and L-arabinose[2] have been proved. Two further advantages afforded by *Pachysolen tannophilus* are the following: conversion of a substrate to ethanol under certain aerobic conditions[3,4] and the possible use of this yeast at relatively high temperatures[5], approximately 37°C.

In a previous paper[6], the influence of environmental variables (pH, temperature and air flow supplied to the medium) on the ethanolic fermentation of glucose was studied. These variables affect ethanol yield and its specific maximum production rate which are the most interesting parameters in order to optimise ethanolic fermentation of sugar solutions. The influence of initial concentrations of glucose and yeast extract on discontinuous ethanolic fermentation using *Pachysolen tanno-*

*philus* is analysed in this study. It was believed that the modification of yeast extract concentration would be of interest since this component seems to supply the vitamins necessary for the microorganism[2].

## EXPERIMENTAL

All experiments were carried out at a laboratory scale in a batch-culture unit described elsewhere[7].

The growth medium composition in $g\,l^{-1}$ was : $MgSO_4$, 1; $KH_2PO_4$, 2; $(NH_4)_2SO_4$, 3; peptone, 3·6 and yeast extract and glucose, both in different concentrations. This medium and the air supplied to the fermentor were sterilized by means of cellulose nitrate filters with a pore size of 0·2 μm. According to a previous paper[6], the values selected for the environmental variables were: 3·5 for the initial pH of the growth medium, 30°C for the temperature and 0·075 v/v/min for the aeration level.

Inocula were prepared and the concentrations of the biomass (expressed in dry weight), of the residual glucose and of the ethanol produced were determined as indicated in the aforementioned paper[6]. In all cases, the concentration of the biomass attained in the fermentor after inoculation, $x_0$, was kept at same level, namely 0·01 $g\,l^{-1}$.

From the experimental results, and through calculus procedures established in the previously mentioned article[6], the values of the following parameters were determined: maximum specific growth ($\mu_m$) and ethanol production ($q_E^{MAX}$) rates, as well as average biomass ($Y_{x/s}$) and ethanol ($Y_{E/s}$) yields.

## RESULTS

Three series of experiments were carried out. In the first, the influence of the initial glucose concentration, $s_0$, was studied: 0, 1, 5, 10, 15, 25, 100, and 200 $g\,l^{-1}$, while the initial concentration of yeast extract was kept constant at 4 $g\,l^{-1}$. Fig. 1 shows the natural logarithm values of the adimensional biomass concentration, $\ln(x/x_0)$, versus time for the six lower concentrations indicated above, while Fig. 3 displays the two highest concentrations. In Table I the variation with respect to time is shown for the concentrations of the residual glucose, $s$, and the ethanol produced, $E$, for the majority of the experiments. In those experiments with $s_0 = 1$ and 10 $g\,l^{-1}$, only the time courses of the biomass concentration were determined and when $s_0 = 0$ the ethanol concentration was negligible.

In the second series, the initial glucose concentration was fixed at 25 $g\,l^{-1}$ while the yeast extract concentration was variable: 0, 1, 2, and 6 $g\,l^{-1}$. The representation of the values of $\ln(x/x_0)$ versus time, appears in Fig. 2 and the values of $s$ and $E$ are shown in Table II.

Finally in the third series, although the initial yeast extract concentration was maintained at 8 $g\,l^{-1}$, the initial concentration of glucose was varied: 100, 150, and 180 $g\,l^{-1}$. The values of $\ln(x/x_0)$ are represented in Fig. 3, while Table III shows the values found for $s$ and $E$.

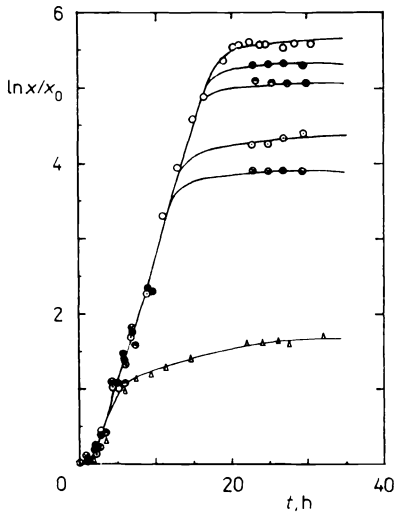


FIG. 1

FIG. 1

Growth curves for $s_0$, g $l^{-1}$: ○ 25, ● 15, ◓ 10, ⊙ 5, ⊗ 1, △ 0; $l_0$ 4 g $l^{-1}$

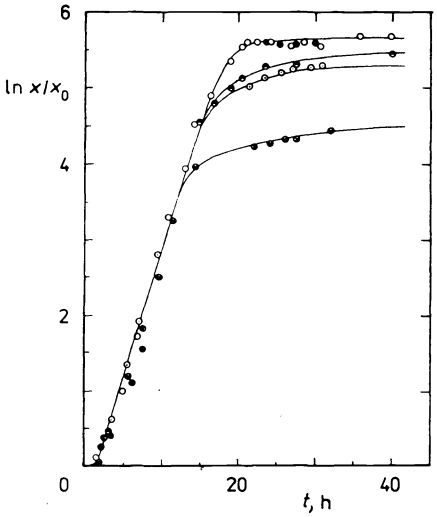


FIG. 2

FIG. 2

Growth curves for $l_0$, g $l^{-1}$: ⊗ 0, ⊙ 1, ◓ 2, ○ 4, ● 6; $s_0$ 25 g $l^{-1}$

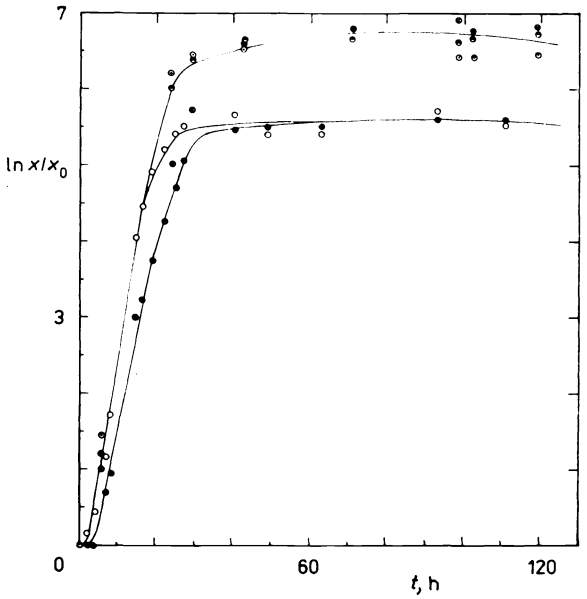


FIG. 3

Growth curves for $s_0$, g $l^{-1}$: ○ 100, ● 200, ⊙ 100, ◓ 150, ⊗ 180; $l_0$, g $l^{-1}$: ○ ● 4, ⊙ ◓ ⊗ 8

## DISCUSSION

### *Cell Growth*

It is clear that for those experiments in which $s_0$ was between 1 and 25 g $l^{-1}$, included in the first series ($l_0 = 4$ g $l^{-1}$), the representation of the values of ln $(x/x_0)$ versus $t$, Fig. 1, the lag phase for all the experiments is very limited, and there is an exponential growth ending in the stationary phase.

In the growth curves of these experiments, there is only a difference in the concentration of the biomass in the stationary phase which occurs once practically all the glucose has been consumed. This points to the fact that glucose is the limiting nutrient. However, in the experiments of the same series in which $s_0$ was raised to

TABLE I

Residual glucose and ethanol produced according to different initial concentration values of glucose, series 1 ($l_0 = 4$ g $l^{-1}$)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $s_0$ 5 g $l^{-1}$ | | | | | |
| $t$, h | 0·0 | 3·0 | 5·0 | 7·0 | 23·0 | 29·5 | | | |
| $s$, g $l^{-1}$ | 5·0 | 4·8 | 4·8 | 4·7 | 0·0 | | | | |
| $E$, g $l^{-1}$ | — | — | 0·007 | 0·008 | 1·6 | 1·3 | | | |
| | | | | $s_0$ 15 g $l^{-1}$ | | | | | |
| $t$, h | 0·0 | 3·0 | 5·0 | 6·0 | 7·0 | 23·0 | 29·5 | | |
| $s$, g $l^{-1}$ | 15·0 | 14·9 | 14·5 | 14·4 | 14·2 | 0·0 | | | |
| $E$, g $l^{-1}$ | — | — | 0·006 | — | 0·007 | 4·7 | 5·0 | | |
| | | | | $s_0$ 25 g $l^{-1}$ | | | | | |
| $t$, h | 0·0 | 5·0 | 11·0 | 15·0 | 19·0 | 20·5 | 24·0 | 30·5 | 40·0 |
| $s$, g $l^{-1}$ | 25·0 | 23·2 | 22·4 | 18·5 | 7·1 | 2·2 | 0·0 | | |
| $E$, g $l^{-1}$ | — | 0·02 | 0·24 | 1·8 | 5·7 | — | 7·1 | 8·8 | 8·2 |
| | | | | $s_0$ 100 g $l^{-1}$ | | | | | |
| $t$, h | 0·0 | 7·0 | 15·5 | 19·5 | 22·5 | 28·0 | 41·0 | 49·5 | 111·5 |
| $s$, g $l^{-1}$ | 100·0 | — | — | — | 78·3 | 64·8 | 48·6 | 37·9 | |
| $E$, g $l^{-1}$ | — | 0·006 | 1·2 | 4·5 | 7·4 | 13·5 | 14·8 | 12·8 | 7·9 |
| | | | | $s_0$ 200 g $l^{-1}$ | | | | | |
| $t$, h | 0·0 | 7·0 | 15·5 | 19·5 | 22·5 | 28·0 | 41·0 | 49·5 | 111·5 |
| $s$, g $l^{-1}$ | 200·0 | — | — | — | — | 183·2 | — | 172·9 | 140·8 |
| $E$, g $l^{-1}$ | — | 0·006 | 0·8 | 1·6 | 2·9 | 6·2 | 11·6 | — | 9·4 |

TABLE II

Residual glucose and ethanol produced according to different initial concentration values of yeast extract, series 2 ($s_0 = 25\ g\ l^{-1}$)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | $l_0$ 0 g $l^{-1}$ | | | | |
| $t$, h | 0·0 | 5·0 | 9·5 | 11·5 | 14·5 | 22·0 | 24·0 |
| $s$, g $l^{-1}$ | 25·0 | — | 24·8 | 24·2 | 23·5 | 18·8 | 18·1 |
| $E$, g $l^{-1}$ | — | 0·008 | 0·012 | 0·062 | 0·12 | 0·66 | 0·74 |
| | | | $l_0$ 1 g $l^{-1}$ | | | | |
| $t$, h | 0·0 | 5·5 | 21·5 | 25·5 | 46·0 | | |
| $s$, g $l^{-1}$ | 25·0 | 24·9 | 14·4 | 9·0 | 0·0 | | |
| $E$, g $l^{-1}$ | — | 0·004 | 3·9 | 4·8 | 4·6 | | |
| | | | $l_0$ 2 g $l^{-1}$ | | | | |
| $t$, h | 0·0 | 17·0 | 19·0 | 20·5 | 23·5 | 44·5 | 65·0 |
| $s$, g $l^{-1}$ | 25·0 | 16·6 | 12·8 | 9·1 | 2·8 | 0·0 | |
| $E$, g $l^{-1}$ | — | 3·0 | — | 5·4 | 7·8 | 8·4 | 6·5 |
| | | | $l_0$ 6 g $l^{-1}$ | | | | |
| $t$, h | 0·0 | 6·0 | 7·5 | 23·5 | 27·5 | 30·0 | |
| $s$, g $l^{-1}$ | 25·0 | 23·4 | 22·7 | 0·0 | | | |
| $E$, g $l^{-1}$ | — | 0·006 | — | 8·8 | 9·0 | 8·6 | |

TABLE III

Residual glucose and ethanol produced according to different initial concentration values of glucose, series 3 ($l_0 = 8\ g\ l^{-1}$)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | $s_0$ 100 g $l^{-1}$ | | | | |
| $t$, h | 0·0 | 6·0 | 24·5 | 30·0 | 43·5 | | |
| $s$, g $l^{-1}$ | 100·0 | 98·2 | 61·3 | 33·6 | 12·2 | | |
| $E$, g $l^{-1}$ | — | 0·026 | 17·4 | 28·4 | 41·8 | | |
| | | | $s_0$ 150 g $l^{-1}$ | | | | |
| $t$, h | 0·0 | 6·0 | 24·5 | 30·0 | 43·5 | 71·5 | 99·0 |
| $s$, g $l^{-1}$ | 150·0 | 149·3 | 120·0 | 99·9 | 51·7 | 47·4 | 41·9 |
| $E$, g $l^{-1}$ | — | 0·023 | 9·8 | 20·9 | 35·7 | 38·9 | 42·0 |
| | | | $s_0$ 180 g $l^{-1}$ | | | | |
| $t$, h | 0·0 | 6·0 | 24·5 | 30·0 | 43·5 | 71·5 | 120·0 |
| $s$, g $l^{-1}$ | 180·0 | — | 154·2 | — | 108·8 | 28·0 | 6·0 |
| $E$, g $l^{-1}$ | — | 0·012 | 9·6 | 13·0 | 31·3 | 62·9 | 56·2 |

100 and 200 g $l^{-1}$, Fig. 3, although the growth curves are analogous to the previous curves, the stationary phase begins when the residual glucose is still at a high level which would suggest that other nutrients or growth-inhibitory phenomena are also present.

From the values of $\ln(x/x_c)$ obtained during the exponential phase of each experiment the values of $\mu_m$ have been determined and are shown in Table IV. In this table, it may be seen that, in the absence of glucose, the value is lower and that a decrease is detected from $s_0 = 25$ g $l^{-1}$, which might indicate the presence of a substrate inhibition at higher glucose concentrations.

The same table shows the biomass yield values which are continually decreasing in the $s_0$ interval of 5–200 g $l^{-1}$. It should be noted that although in the control experiment, which was performed in the absence of glucose, the biomass concentration corresponding to the stationary phase is very low (Fig. 1) this value was also considered for the purpose of obtaining the biomass yield.

The growth curves from the experiments in the series designed to study the influence of $l_0$ (Fig. 2) show a common exponential phase, while the curves differ considerably in the biomass concentration values obtained in the stationary phase. It would seem, therefore, that there is no significant influence of $l_0$ on the $\mu_m$ value, while $l_0$ does indeed influence the biomass yield levels, these being lower for $l_0$ equal to 0 and 1 g $l^{-1}$, $Y_{x/s} = 0{\cdot}08$ g $g^{-1}$, than for $l_0$ between 2 and 6 g $l^{-1}$, $Y_{x/s} = 0{\cdot}11$ g $g^{-1}$. Moreover for $l_0$ lower than 2 g $l^{-1}$, the stationary phase begins when residual glucose is even more significant. This suggests that in those experiments, the yeast extract may have been the limiting nutrient.

This aspect may also be deduced from the study on the third series in which the concentration of yeast extract was doubled, $l_0 = 8$ g $l^{-1}$, for initially high concentrations of glucose. Thus, in the growth curves represented in Fig. 3, it is clear that

TABLE IV
Maximum specific growth rates and biomass yields for the first series ($l_0 = 4$ g $l^{-1}$)

| $s_0$, g $l^{-1}$ | $\mu_m$, $h^{-1}$ | $Y_{x/s}$, g $g^{-1}$ |
|---|---|---|
| 0 | 0·23 | — |
| 1 | 0·33 | — |
| 5 | 0·33 | 0·17 |
| 10 | 0·33 | — |
| 15 | 0·33 | 0·16 |
| 25 | 0·33 | 0·11 |
| 100 | 0·31 | 0·08 |
| 200 | 0·24 | 0·07 |

the biomass concentrations in the stationary phase are considerably higher in experiments with an initial double yeast extract concentration, in a way that is virtually independent of the value for $s_0$. Moreover, the values for residual glucose at the beginning of the stationary phase are much lower in experiments with a higher value for $l_0$. It is this circumstance that determines the relative independence of the biomass yield from $l_0$ in the 4 to 8 g $l^{-1}$ interval for values of $s_0$ between 100 and 200 g $l^{-1}$, thereby obtaining a value of $Y_{x/s} = 0{\cdot}08$ g $g^{-1}$ for $s_0 = 100$ and $l_0 = 8$ g $l^{-1}$, and a value of $Y_{x/s} = 0{\cdot}07$ g $g^{-1}$ for the experiments when $l_0 = 8$ g $l^{-1}$ and $s_0 = 150$ and 180 g $l^{-1}$.

*Ethanol Production*

To determine the ethanol yield, $Y_{E/s}$, the values for ethanol concentrations, $E$, are represented against the values for glucose consumed simultaneously, $(s_0 - s)$. Thus, Figs 4 and 6 show the representations corresponding to each of the series performed, and from these figures it may be observed that in all cases a linear relationship is feasible between both variables where their slopes correspond to the value of $Y_{E/s}$.

In the first series, Fig. 4, an average value for $Y_{E/s}$ of 0·38 (g ethanol) . (g glucose)$^{-1}$ independent of $s_0$ has been obtained for values of $s_0$ between 5 and 200 g $l^{-1}$ although for the two experiments with a higher value of $s_0$ only the times of low glucose

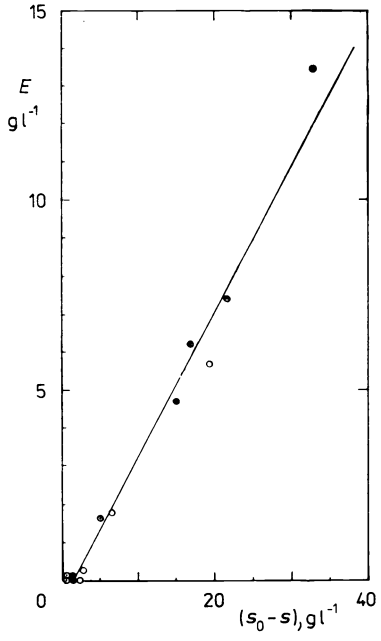


FIG. 4

Ethanol produced versus glucose consumed for the first series. $l_0$ 4 g $l^{-1}$; $s_0$, g $l^{-1}$: ⊙ 5, ● 15, ○ 25, ◓ 100, ⊗ 200

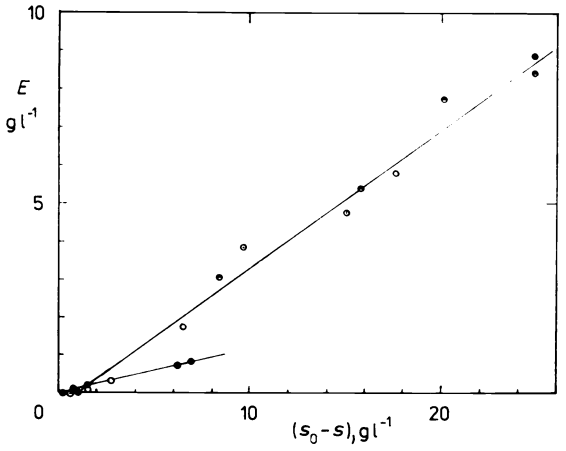


FIG. 5

Ethanol produced versus glucose consumed for the second series. $l_0$, g $l^{-1}$; $s_0$ 25 g $l^{-1}$: ⊗ 0, ⊙ 1, ◓ 2, ○ 4, ● 6

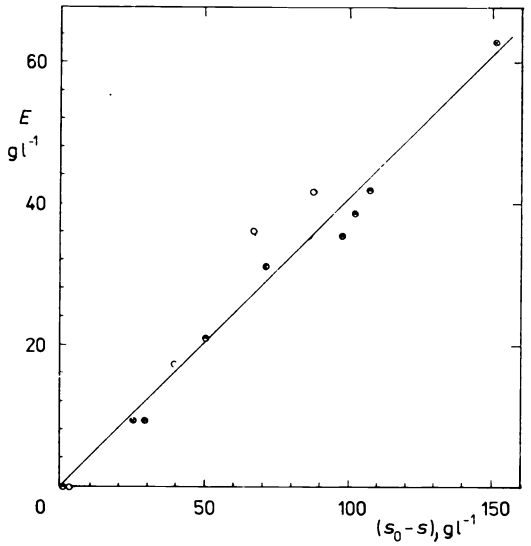


FIG. 6

Ethanol produced versus glucose consumed for the third series. $l_0$ 8 g $l^{-1}$; $s_0$, g $l^{-1}$: ○ 100, ◓ 150, ⊙ 180

consumption may be considered (lower than 35 g $l^{-1}$). In the second series, Fig. 5, an average value of 0·36 g $g^{-1}$ may be obtained for values of $l_0$ between 1 and 6 g $l^{-1}$ while in the experiment carried out in the absence of yeast extract, the yield decreases considerably to 0·11 g $g^{-1}$. This decrease proves the importance of this nutrient in ethanol production, although for $s_0 = 25$ g $l^{-1}$ it seems that 1 g $l^{-1}$ of yeast extract is sufficient for the ethanol yield to remain practically unaltered.

In the third series, $l_0 = 8$ g $l^{-1}$, Fig. 6, an average value of 0·41 g $g^{-1}$ is obtained. This value is slightly higher than the 0·38 g $g^{-1}$ obtained for the first series, $l_0 = 4$ g $l^{-1}$, although the most outstanding feature is that this yield is maintained with much higher glucose consumption. This determines that ethanol concentrations of up to 63 g $l^{-1}$ are obtained in the experiment with a higher $s_0$ value, far higher than in the experiments in the first series.

Although the value of 0·41 g $g^{-1}$ already implies a remarkable ethanol yield, it only corresponds to 80% of the theoretical yield of 0·511 g $g^{-1}$. In order to partially justify this difference a corrected ethanol yield may be calculated, $Y'_{E/s}$, where the glucose converted into the biomass is taken into account and if it is admitted that glucose is the only carbon source, we would obtain the following expression:

$$Y'_{E/s} = \frac{E}{(s_0 - s)\left[1 - cY_{x/s}(M_s/6A_c)\right]} = \frac{E}{s'}$$

For the evaluation of the above the carbon mass fraction in the biomass produced must be known. Bearing this in mind, an elemental analysis was carried out on the biomass and the following composition in mass fractions was determined: C = 0·446, H = 0·067, N = 0·0837, the remaining fraction being attributable to oxygen. The corresponding formula, therefore, would be: $CH_{1.81}N_{0.16}O_{0.68}$.

The representation of values for $E$ versus the denominator of the previous equation, $s'$, calculated with the biomass yields mentioned earlier, is shown in Figs 7 and 8 as an example for two of the experimental series. Linear relations may be permitted, with slopes determining values for $Y'_{E/s}$ of 0·42 g $g^{-1}$ in the first series, 0·41 g $g^{-1}$ in the second (where, with $l_0 = 0$, the value is reduced to 0·12 g $g^{-1}$) and of 0·44 g $g^{-1}$ in the third series. The latter value represents 86% of the theoretical value and the difference may be attributed to different causes such as cell maintenance, the formation of products other than ethanol, metabolic consumption of ethanol or loss through evaporation.

Values of $q_E^{MAX}$ have also been determined and are shown in Table V. It can be observed that in the first series from $s_0 = 25$ g $l^{-1}$ $q_E^{MAX}$ remains practically constant while in the other two series it again becomes clear that yeast extract is of major importance. Thus, in the absence of yeast extract, the value for $q_E^{MAX}$ is very low and on doubling the concentration, at high values for $s_0$, there is a significant increase with regard to the first series.

In order to confirm these values and to establish a relationship between ethanol production and cell growth, representations of $E$ versus the biomass produced

TABLE V
Maximum specific ethanol production rates for all three series

| $s_0$, g l$^{-1}$ | $l_0$, g l$^{-1}$ | $q_E^{MAX}$, g g$^{-1}$ h$^{-1}$ | $\mu_m Y_{E/x}$, g g$^{-1}$ h$^{-1}$ |
|---|---|---|---|
| 5 | 4 | 0·2 | 0·7 |
| 15 | 4 | 0·3 | 0·7 |
| 25 | 4 | 0·8 | 1·0 |
| 100 | 4 | 0·8 | 0·9 |
| 200 | 4 | 0·9 | 1·1 |
| 25 | 0 | 0·1 | 0·1 |
| 25 | 1 | 0·6 | 1·0 |
| 25 | 2 | 1·0 | 1·0 |
| 25 | 6 | 0·8 | 1·0 |
| 100 | 8 | 1·8 | 1·8 |
| 150 | 8 | 2·0 | 1·5 |
| 180 | 8 | 1·9 | 1·5 |

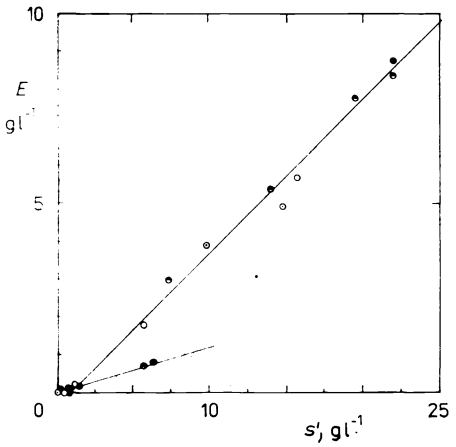


FIG. 7
Ethanol produced versus a fraction of the glucose consumed for the second series. $l_0$, g l$^{-1}$; $s_0$ 25 g l$^{-1}$: ⊗ 0, ⊙ 1, ◓ 2, ○ 4, ● 6

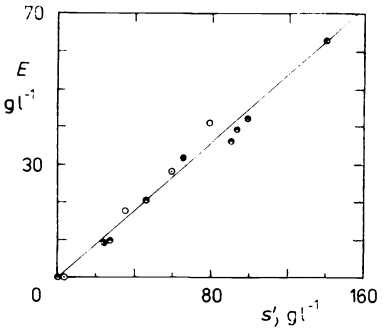


FIG. 8
Ethanol produced versus a fraction of the glucose consumed for the third series. $l_0$ 8 g l$^{-1}$; $s_0$, g l$^{-1}$: ⊙ 100, ◓ 150, ⊗ 180

were drawn. An example of the representation corresponding to the first two series is given in Figs 9 and 10. It may be deduced that a linear relationship may be drawn which suggests that ethanol production is closely linked to cell growth and the slope, $Y_{E/x}$, allows the estimation of the value for specific rate of ethanol production in the exponential growth phase through the product $\mu_m Y_{E/x}$. The corresponding values for the three series are shown in Table V where they can be seen that they are in same order of the values for $q_E^{MAX}$.

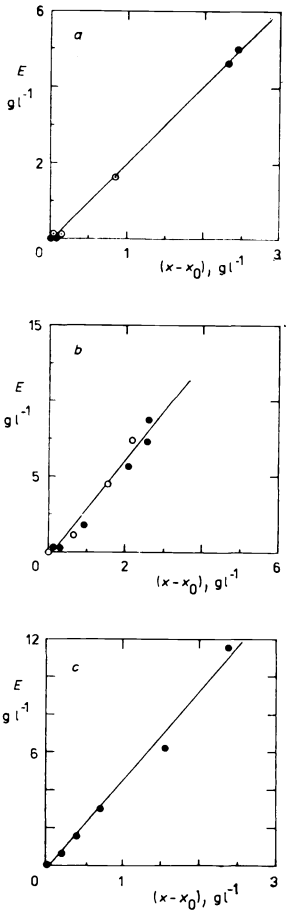


FIG. 9

Ethanol produced versus biomass formed for the first series. $l_0$ 4 g l$^{-1}$; $s_0$, g l$^{-1}$: *a* ⊙ 5, ● 15; *b* ● 25, ○ 100; *c* ● 200

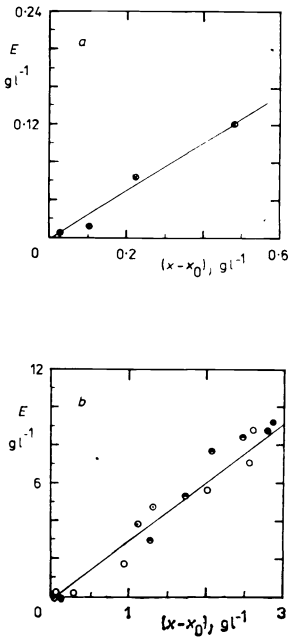


FIG. 10

Ethanol produced versus biomass formed for the second series. $s_0$ 25 g l$^{-1}$; *a* $l_0$ = = 0 g l$^{-1}$; *b* $l_0$, g l$^{-1}$: ⊙ 1, ◓ 2, ○ 4, ● 6

## CONCLUSIONS

The study of ethanolic fermentation of glucose solutions with *Pachysolen tannophilus* by modifying the initial glucose and yeast extract concentrations has stressed the importance of yeast extract both in cell growth and in ethanol production. This means that the dosage of yeast extract in the fermentation of hydrolyzed cellulose residues should be appropriately optimized.

In the concentration intervals studied, values of up to $0{\cdot}33\ h^{-1}$ were obtained for the specific maximum growth rate which are slightly higher than the values 0·29 and $0{\cdot}24\ h^{-1}$ obtained during fermentation under aerobic conditions with xylose solutions[8,9]; values in the order of $0{\cdot}4\ g\ g^{-1}$ were obtained for ethanol yield which are also higher than those indicated[8] as the maximum for xylose as a substrate of $0{\cdot}34\ g\ g^{-1}$ as well as values up to $2\ g\ g^{-1}\ h^{-1}$ for specific maximum ethanol production rates far higher than those for xylose, $0{\cdot}13\ g\ g^{-1}\ h^{-1}$, obtained by Slininger et al.[8] and also higher than those indicated for glucose[10] namely 0·22 and $0{\cdot}1\ g\ g^{-1}\ h^{-1}$ under aerobic and anaerobic conditions, respectively.

Moreover, the comparison of values obtained with those afforded by *Saccharomyces cerevisiae* (0·4 to $0{\cdot}45\ h^{-1}$ for $\mu_m$ (ref.[11]) and $0{\cdot}5\ g\ g^{-1}$ for $Y_{E/s}$ and 0·9 g . . $g^{-1}\ h^{-1}$ for $q_E^{MAX}$, the latter obtained in fermentation of solutions of $50\ g\ l^{-1}$ of glucose[12]) suggests that the lower ethanol yield may be compensated by the higher value of $q_E^{MAX}$ and fundamentally by the possibility of effecting fermentation with xylose.

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## SYMBOLS

| | |
|---|---|
| $A_c$ | Carbon atomic weight |
| $E$ | Ethanol concentration, $g\ l^{-1}$ |
| $c$ | Carbon mass fraction in the biomass |
| $l_0$ | Initial yeast extract concentration, $g\ l^{-1}$ |
| $M_s$ | Glucose molecular weight |
| $q_E^{MAX}$ | Maximum specific ethanol production rate, (g ethanol) . $(g\ biomass)^{-1}\ (h)^{-1}$ |
| $s$ | Residual glucose concentration, $g\ l^{-1}$ |
| $s'$ | A fraction of the glucose consumed as defined in the equation in the text, $g\ l^{-1}$ |
| $s_0$ | Initial glucose concentration, $g\ l^{-1}$ |
| $t$ | Time, h |
| $x$ | Biomass concentration, $g\ l^{-1}$ |
| $x_0$ | Initial biomass concentration, $g\ l^{-1}$ |
| $Y_{E/s}$ | Average ethanol yield, (g ethanol) . $(g\ glucose)^{-1}$ |
| $Y'_{E/s}$ | Average corrected ethanol yield, g ethanol . $(g\ glucose)^{-1}$ |
| $Y_{E/x}$ | Slope of the graph of $E$ versus $(x - x_0)$, (g ethanol) . $(g\ biomass)^{-1}$ |
| $Y_{x/s}$ | Average biomass yield, (g biomass) . $(g\ glucose)^{-1}$ |
| $\mu_m$ | Maximum specific growth rate, $h^{-1}$ |

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