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ETHANOLIC FERMENTATION OF D-XYLOSE AND PINE WOOD HYDROLYZATE BY THE YEAST *Pachysolen tannophilus*

A thesis presented in partial fulfilment of the requirements for the degree of **Doctor of Philosophy** in **Biotechnology** at **Massey University**

TZE SEN WONG

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ABSTRACT

This thesis reports a study of the ethanolic fermentation of D-xylose and wood hydrolyzate to ethanol by the yeast Pachysolen tannophilus with a view to developing an effective use of renewable hemi-cellulose hydrolysis products from New Zealand forest biomass residues.

Initial work briefly addressed the problem of finding a suitable yeast from natural habitats suitable for the fermentation. Soon after that work commenced literature reports suggested that preliminary conversion of pentoses by enzymatic means was a possibility. Consequently, this aspect of conversion was considered and rejected. One reason for this was that literature was drawing attention to the pentose fermenting characteristics of Pachysolen tannophilus.

Laboratory scale studies demonstrated the yeast Pachysolen tannophilus to be capable of fermenting the hexose and pentose sugars present in the hydrolyzate. The yeast's specific growth rate in the hydrolyzate could be improved by neutralizing the inhibitory substances with 2 g/l of anhydrous sodium sulphite. Ethanol has an inhibitory effect on growth but can also be readily assimilated by the yeast.

Fermentation studies with gyration speeds of 50, 100 and 200 r.p.m. showed that oxygen was a critical parameter affecting growth and ethanol production. Batch fermentation experiments were pursued to examine this oxygen phenomenon more closely. Cell growth, substrate uptake rate and culture pH responded strongly to the supply of oxygen. However, production of ethanol accompanied cell growth only in late "exponential" phase.

Fermentation characteristics were established under continuous culture at an aeration rate of 0.37 l/l.min and values obtained were as follows; maximum specific growth rate, 0.046 h^{-1}; biomass yield, 0.04 g/g; ethanol yield, 0.17 g/g; Ks value, 13 g/l and Ki values, 0.5 g/l.

A redox potential controlled chemostat study revealed that steady-state culture poised at -50 mV exhibited a 55% increased ethanol concentration and 43% decreased xylitol concentration over the value observed without redox control.

With a knowledge of D-xylose fermentation as established in these batch and chemostat experiments, it was possible to consider more detailed aspects of the fermentation which would be applicable to process development. Questions addressed included which strain of Pachysolen tannophilus should be used, what quantity of inoculum was necessary, what interactions existed between fermentation variables. Statistically designed experiments were employed to answer these questions. Empirical models so developed revealed that ethanol yield has a linear relationship with initial substrate concentration. These models have given some insight into how environmental factors affect the ethanolic fermentation by this yeast and have also indicated the optimal conditions required for an effective fermentation of wood pentoses.

These important fermentation process variables were established and are expected to be useful in moving the process from laboratory scale as carried out here to a pilot plant scale of operations. The values established were temperature, 28° or lower; initial medium pH for ethanol production, 5.6 to 5.8; substrate concentration used can be up to 80 g/l of pentoses; minimum inoculum density, 5.5 g/l dry weight cells and NRRL Y-2461 was recommended as the best strain to achieve the fermentation. The pre-treatment of the prehydrolyzate by 2 g/l of anhydrous sodium sulphite was highly desirable in order to enhance growth and fermentation rates.

The research has shown that Pachysolen tannophilus is capable of fermenting pentose fraction of wood hydrolyzate and that the optimal conditions for this fermentation will lead to significant utilization of wood sugar. However, in the completely mixed reactor systems used in these experiments, the ethanol yields obtained were not as attractive as those observed for hexose fermentations under similar conditions. This, it is felt, points to the greater difficulty the yeast experiences in fermenting pentoses and it also suggests the need to investigate the value of other reactor formats at some future date.

17 December , 1984.

This is to certify that the work on which the thesis, *ETHANOLIC FERMENTATION OF D-XYLOSE AND PINE WOOD HYDROLYZATE BY THE YEAST Pachysolen tannophilus,* is based has not been accepted in whole or in part for any other degree or diploma and all the help and assistance received in the research on which the thesis is based, are fully acknowledged.

Tze Sen Wong.
Ph. D. Candidate.

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ABBREVIATIONS AND SYMBOLS

g	gram or gravitational force under centrifugation
h	hour
kg	kilogram
l	litre
M	molar or mole
mg	milli gram
min	minute
ml	milli litre
mM	milli mole
mV	milli volt
ppb	part per billion (i.e. μg/l)
s	second
r.p.m.	revolution per minute
vvm	gas volume/medium volume/min (l/l.min)
v/v	volume/volume (ml/l)
w/v	weight/volume (g/l)

A.R.	analytical reagent
ATP	adenosine-5'-triphosphate
AW-DMCS	acid washed - dimethyldichlorosilane
BOD	biochemical oxygen demand (g/l)
COD	chemical oxygen demend (g/l)
D	dilution rate (h^{-1})
DEAE-	diethylaminoethyl-
DF	degrees of freedom
DOT	dissolved oxygen tension
dP/dt	rate of production formation (g/l.h)
dS/dt	rate of substrate consumption (g/l.h)
E	ethanol concentration (g/l)
F	F-ratio of mean sums of squares
FFAP	free fatty acid phase
F_{1of}	F-ratio of MSLF to MSPE
Ki	ethanol inhibition constant for growth (g/l)
Km	the Michaelis constant

Ks	saturation constant
log	logarithm to base 10
ln	logarithm to base e
m	maintenance energy coefficient (g substrate/g dry cell.h)
MRS	de Man, Rogosa, Sharpe agar or broth
MS	mean sum of squares
MSLF	mean sum of squares due to lack of fit
MSPE	mean sum of squares due to pure error
MSRG	mean sum of squares due to regression
MSRS	mean sum of squares due to residual
N	number of replicates
NADP	nicotinamide adenine dinucleotide phosphate
N_c	number of centre points in experimental design
N_f	number of factorial points in experimental design
N_s	number of `star' points in experimental design
Qp	specific product formation rate (g/g.h)
Qs	specific substrate consumption rate (g/g.h)
So	initial sugar (D-xylose) concentration (g/l)
Sx	steady state value of D-xylose concentration (g/l)
Sxo	xylitol concentration (g/l)
SS	sum of square
STDEV	standard deviation
TCA	tricarboxylic acid cycle
W	dry weight of cells (g/l)
X	biomass concentration (g/l)
Y	response variable or observed growth yield (g cells/g substrate)
Yg	true growth yield (g cells/g substrate)
$\hat{Y}$	predicted value of response variable being modelled
Y p/s	product yield
Y p/x	product yield
Y x/s	biomass yield
Y xo/s	xylitol yield
Y xo/x	xylitol yield

$>$	is greater than
$<$	is less than
α	coded distance from origin of `star' or axial point in a central composite or central composite rotatable design
β	represents the coefficient in the experimental design
β_o	is the Y-intercept or constant term
μ	spcific growth rate $(1/X.\ dX/dt)$ (h^{-1})
μ_{max}	spcific growth rate $(1/X.\ dX/dt)$ (h^{-1})
^{o}	represents degrees temperature expressed on Celsius scale

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