High gravity extractive fermentation for enhanced productivity of bioethanol

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Abstract

Bioethanol is a renewable alcohol fuel produced from sugary substrates via fermentation processes. Accumulation of ethanol in the fermentation broth inhibits cell growth and further production of ethanol. Recovering ethanol from a dilute broth is expensive. Ethanol inhibition of fermentation may be reduced by continuously removing it as it is formed.

This work focussed on production of bioethanol from glucose using the anaerobic bacterium *Zymomonas anaerobia*. This bacterium produces ethanol more rapidly than does the conventional yeast fermentation. The aim was to assess the impact of continuous in-situ removal of ethanol on the productivity of batch and continuous high-gravity fermentations. High-gravity fermentations use a medium with a high concentration of sugar to reduce production volume (bioreactor size) and potentially achieve a high productivity of ethanol, if the ethanol concentration in the broth can be kept to below inhibitory levels.

First, the batch fermentation was characterized for glucose tolerance, ethanol tolerance, optimal production temperature and biocompatibility with solvents and adsorbents that could be used for in-situ removal of ethanol. High gravity media containing 50–300 g L^{-1} glucose were used to characterize batch and continuous fermentations with a view to identifying the best fermentation conditions for a detailed study. The optimal fermentation temperature was found to be 35 °C and the maximum tolerable initial glucose (i.e. without causing substrate inhibition) was 150 g L^{-1}.

In continuous high-gravity fermentations, six different dilution rates (D = 0.05–0.30 h^{-1}) were tested, but steady-state operation proved to be impossible at the lowest dilution rate: the fermentation showed a highly consistent oscillatory behaviour that was ascribed to ethanol toxicity. Use of higher dilution rates could overcome oscillations by washing out the ethanol from the bioreactor, but this reduced ethanol productivity as glucose and biomass also washed out. Strategies for removing ethanol in-situ while operating at such a dilution rate as to achieve a high ethanol productivity, were assessed by using liquid-liquid extraction and adsorption on polymer resins as methods for removing ethanol as it was being produced.

In the absence of in-situ removal of ethanol, the lowest operable steady-state dilution rate (i.e. without oscillations) was 0.15 h^{-1}. With in-situ removal of ethanol, the dilution rate for stable steady state operation could be reduced to 0.05 h^{-1}. At a dilution rate of 0.15 h^{-1}, the steady-state ethanol concentration was 42.5 g L^{-1} and the biomass concentration was 1.49 g L^{-1}. In the absence of in-situ ethanol removal, the ethanol concentration, but not ethanol productivity, was highest at a dilution rate of 0.3 h^{-1} although much residual glucose remained.

In in-situ batch extractive fermentations, all extraction solvents tested improved biomass concentration, glucose consumption and ethanol concentration relative to control, but iso-octadecanol was clearly the most effective solvent. For batch in-situ extractive fermentation with iso-octadecanol, the ethanol yield on glucose was 0.485±0.005 g g^{-1}, or comparable to to a yield of 0.468±0.005 g g^{-1} for the control culture, but the ethanol productivity was distinctly higher than for the control culture. Of the various polymer resins tested in batch fermentations for in-situ removal of ethanol by adsorption, Dowex Optipore L-493 appeared to be somewhat better than the control (i.e. no resin).

The best extraction solvent (i.e. iso-octadecanol) and the best adsorption resin (i.e. Dowex Optipore L-493) were separately assessed for ethanol removal in continuous fermentations. Continuous removal of ethanol both by adsorption and solvent extraction allowed a steady-state operation of the continuous fermentations at a dilution rate of 0.05 h^{-1} — the dilution rate at which steady-state operation had proved impossible in control fermentation (i.e. without in-situ removal of ethanol). This confirmed the mechanism used to explain the oscillatory behaviour of the fermentation and showed that in-situ ethanol removal permitted steady-state operation at dilution rates that would not allow such operation in the absence of ethanol removal. At a dilution rate of 0.05 h^{-1}, an extraction solvent flow rate of 300 mL h^{-1} provided the highest total ethanol productivity and ethanol yield on glucose while keeping the solvent use to a minimum.

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Table of Contents

List of Figures

List of Tables

Abbreviations

A	-	Cross sectional area of the column (m^2)
C_{Aq}	-	Aqueous phase concentration of ethanol (g L^{-1})
C_e	-	Equilibrium concentration of ethanol in glucose solution (g L^{-1})
D	-	Dilution rate (h^{-1})
D_p	-	Particle size (m)
E	-	Maximum ethanol concentration (g L^{-1})
E_A	-	Steady state ethanol concentration in aqueous phase (g L^{-1})
E_S	-	Steady state ethanol concentration in solvent phase (g L^{-1})
Fs	-	Flow rate of solvent (mL h^{-1})
g	-	Acceleration due to gravity (m s^{-2})
H	-	Height of expanded resin bed (m)
H_o	-	Bed height of original settled resin at superficial flow velocity of zero
K_a	-	Distribution coefficients of solute a in the solvent
K_b	-	Distribution coefficients of solute b in the solvent
K_d	-	Partition coefficient
K_F	-	Freundlich constant
K_L	-	Langmuir constant
K_r	-	Partitioning coefficient of ethanol on resin
K_s	-	Limiting substrate concentration (g L^{-1})
L	-	Specific loading
n	-	Freundlich coefficient
P_E	-	Ethanol productivity (g L^{-1} h^{-1})
P_{EA}	-	Steady state ethanol productivity in the aqueous phase ($gL^{-1}h^{-1}$)
P_{ES}	-	Steady state ethanol productivity in the solvent phase ($gL^{-1}h^{-1}$)
P_{ET}	-	Total ethanol productivity (g L^{-1} h^{-1})
P_x	-	Biomass productivity (g L^{-1} h^{-1})
q_e	-	Equilibrium adsorption capacity of ethanol on the adsorbent (g kg^{-1})
Q_F	-	Medium flow rate (mL h^{-1})
Q_L	-	Flow rate (mL h^{-1})

Q_S	-	Solvent flow rate (mL h^{-1})
q_m	-	Maximum adsorption capacity of ethanol on the adsorbent (g kg^{-1})
q_p	-	Average specific ethanol production rate (g g^{-1} h^{-1})
q_s	-	Average specific glucose uptake rate (g g^{-1} h^{-1})
R	-	Ideal gas constant (J mol^{-1} K^{-1})
R^2	-	Regression coefficient
S	-	Glucose concentration (g L^{-1})
S_o	-	Initial substrate concentration (g L^{-1})
T	-	Absolute temperature (K)
t	-	Time (h)
t_0	-	Initial time (h)
t_f	-	Time at equilibrium (h)
V_L	-	Liquid volume in the reactor (L)
V_s	-	Volume of solvent (L)
X_{max}	-	Biomass concentration (g L^{-1})
X_r	-	Mass of resin per unit volume of aqueous phase (g L^{-1})
W_o	-	Weight of aluminium pan and sample before drying (g)
W_f	-	Weight of aluminium pan and sample after drying (g)
$Y_{p/s}$	-	Ethanol yield on substrate (g g^{-1})
$Y_{x/s}$	-	Biomass yield on substrate (g g^{-1})
μ	-	Specific growth rate (h^{-1})
ε	-	Void volume of expanded bed
σ_L	-	Liquid density (kg m^{-3})
ε_m	-	Minimum bed void volume of a settled bed of spherical particles (m)
σ_p	-	Solid density (kg m^{-3})
$[E]_{aq}$	-	Ethanol concentration in the aqueous phase (g L^{-1})
$[E]_{org}$	-	Ethanol concentration in the organic phase (g L^{-1})
μ_{max}	-	Maximum specific growth rate (h^{-1})
ΔG	-	The adsorption Gibbs free energy (kJ/mol)

ρ	-	Density (kg/m^3)
β	-	Selectivity