Use of ultrasound in enhancing productivity of biotechnological processes

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Abstract

This study focused on identifying optimum sonication regimens (e.g. intensity, duty cycles) that may intensify bioprocesses without damaging the biocatalyst. Possible mechanisms of productivity enhancement in various biotechnology processing scenarios were investigated. Three model processes were used: 1) production of bioethanol from lactose by fermentation with the yeast *Kluyveromyces marxianus*; 2) β-galactosidase catalyzed hydrolysis of lactose in a homogeneous cell-free system; and 3) hydrolysis of soluble and insoluble particulate cellulose of various sizes, catalyzed by soluble cellulase. The above processes involved: 1) conversion of a soluble substrate by a live catalyst in the presence of gas-liquid mass transfer; 2) a cell-free homogeneous bioreaction system; and 3) a heterogeneous reaction system involving substantial solid-liquid mass transfer limitations depending on the size of the substrate (i.e. soluble and insoluble particulate cellulose).

Low intensity ultrasound (11.8 W cm^{-2} sonication power at the sonotrode tip), enhanced the ethanol productivity of the batch fermentation process. At the specified sonication intensity a duty cycle of 20% was found to be optimal. A duty cycle of 40% adversely affected the fermentation. With the best duty cycle of 20%, the final ethanol concentration was 5.2±0.68 g L^{-1}, or nearly 3.5-fold that of the control fermentation. The productivity enhancing effect of sonication was attributed to a possible improved desorption of carbon dioxide from the fermentation broth. Ultrasound may also have facilitated transport of lactose into the cell by affecting cell permeability. While ultrasound apparently enhanced desorption of carbon dioxide, it also damaged yeast enzymes such as β-galactosidase and this may explain why a 40% duty cycle had an adverse impact on the fermentation. Although at the highest duty cycle of 40% sonication reduced cell growth, cell viability remained high at ≥70% during most of the fermentation. In continuous fermentations, sonication always enhanced the steady-state biomass concentration and ethanol concentration at all dilution rates tested relative to the corresponding controls.

Ultrasound effectively influenced enzyme-substrate binding/unbinding for β-galactosidase mediated hydrolysis of lactose in a cell-free system. A short irradiation pulse (i.e. 10% duty cycle), applied at the highest irradiation power (11.8 W cm^{-2}),

improved the initial hydrolysis rate, by nearly 1.4-fold relative to control. This effect of ultrasound was possibly due to its accelerative effect on collision frequency of the enzyme and substrate molecules as a consequence of the microturbulence caused by sonication.

The cellulase-mediated hydrolysis of soluble cellulose as well as particulate cellulose was enhanced by sonication at a 10% duty cycle and power intensity of 11.8 W cm^{-2}, but prolonged sonication adversely impacted the enzyme stability at a constant temperature of 50 °C relative to control.

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Abbreviations

A	Cross-sectional area (m^2)
a_L	Interfacial area per unit liquid volume (m^{-1})
C^*	Dissolved oxygen saturation concentration (mol L^{-1})
C_0	Dissolved oxygen concentration in bulk liquid phase (% sat or mol L^{-1})
C_L	Actual instantaneous concentration of dissolved oxygen in the broth (mol L^{-1})
CMC	Carboxymethyl cellulose
C_p	Dissolved oxygen concentration measured by probe (mol L^{-1} or % of air saturation)
D	Dilution rate (h^{-1})
E_o	Initial enzyme concentration (g L^{-1})
E_{max}	Maximum ethanol concentration (g L^{-1})
f	Frequency (s^{-1})
H	Henry's law constant
I	Sonication intensity (W cm^{-2})
k_L	Mass transfer coefficient (m s^{-1})
k_La_L	Volumetric mass transfer coefficient (s^{-1})
K_m	Michaelis constant (g L^{-1})
k_p	Response coefficient of oxygen probe (s^{-1})
K_s	Saturation constant for substrate (g L^{-1})
P	Power input (W)
P_E	Ethanol productivity (g L^{-1} h^{-1})
P_{ir}	Irradiation power of ultrasound (W)
P_x	Biomass productivity (g L^{-1} h^{-1})
q_p	Average biomass specific ethanol production rate (g g^{-1} h^{-1})
q_s	Average biomass specific lactose uptake rate (g g^{-1} h^{-1})
S	Substrate concentration (g L^{-1})
S_e	Substrate concentration in the reactor at steady state (g L^{-1})
S_o	Initial substrate concentration (g L^{-1})
t	Time (s)

t_m	Mass transfer time (s)
V_i	Initial reaction rate (M s^{-1})
V_L	Liquid volume in the reactor (m^3)
V_{max}	Maximum reaction rate (M s^{-1})
X_{max}	Biomass concentration (g L^{-1})
$Y_{p/s}$	Ethanol yield on substrate (g g^{-1})
$Y_{x/s}$	Biomass yield on substrate (g g^{-1})
τ_p	Probe response time (s)
μ	Liquid viscosity (mNs/m^2)
μ	Specific growth rate (h^{-1})
μ_{max}	Maximum specific growth rate (h^{-1})
ρ	Density (kg/m^3)