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Mechanistic insight into ultrasound induced enhancement of simultaneous saccharification and fermentation of *Parthenium hysterophorus* for ethanol production

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ABSTRACT

This paper presents investigations into mechanism of ultrasound assisted bioethanol synthesis using *Parthenium hysterophorus* biomass through simultaneous saccharification and fermentation (SSF) mode. Approach of coupling experimental results to mathematical model for SSF using Genetic Algorithm based optimization has been adopted. Comparison of model parameters for experiments with mechanical shaking and sonication (10% duty cycle) give an interesting mechanistic account of influence of ultrasound on SSF system. A 4-fold rise in ethanol and cell mass productivity is seen with ultrasound. The analysis reveals following facets of influence of ultrasound on SSF: increase in Monod constant for glucose for cell growth, maximal specific growth rate and inhibition constant of cell growth by glucose and reduction in specific cell death rate. Values of inhibition constant of cell growth by ethanol (K_{3E}), and constants for growth associated (a) and non-growth associated (b) ethanol production remained unaltered with sonication. Beneficial effects of ultrasound are attributed to enhanced cellulose hydrolysis, enhanced trans-membrane transport of substrate and products as well as dilution of the toxic substances due to micro-convection induced by ultrasound. Intrinsic physiological functioning of cells remained unaffected by ultrasound as indicated by unaltered values of K_{3E}, a and b.

1. Introduction

Bioethanol production from lignocellulosic biomass has been a highly active research area for past several years, as ethanol has shown high promise as an alternate liquid transportation fuel as well as oxygenate blend for gasoline. The conventional process for bioethanol production has two steps, viz. pretreatment and acid/enzymatic hydrolysis of lignocellulosic biomass followed by fermentation of the acid and/or enzymatic hydrolyzate. The cost of production of bioethanol is a major function of cost of fermentation substrate as well as the operating cost of the process. Lignocellulosic biomass available abundantly in the form of agro-residues, forest-residues, and waste biomass (weed/grass) forms a potential low-cost feedstock for bioethanol. Some typical examples of waste biomass whose carbohydrate moieties have been used for bioethanol production are *Saccharum spontaneum* [1], *Lantana camara* [2] and *Prosopis juliflora* [3]. In order to intensify the bioethanol productivity while reducing the cost of production, the process of simultaneous saccharification and fermentation (SSF) has also been extensively investigated. This process has distinct advantages of milder operating conditions, and requirement of a single fermentor vessel that combines the two steps of hydrolysis and fermentation mentioned above. In this process, hexose sugars released from enzymatic hydrolysis of cellulose in the biomass are simultaneously consumed by fermenting microorganisms. The enzymatic hydrolysis of cellulose itself is a two-step process, in which first cellulase hydrolyzes the cellulose into

Abbreviations: CMCase, carboxymethylcellulase; GA, Genetic Algorithm; GC, gas chromatography; HPLC, high performance liquid chromatography; MTCC, microbial type culture collection; RI, refractive index; SHF, separate hydrolysis and fermentation; SSF, simultaneous saccharification and fermentation; TRS, total reducing sugar.

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Nomenclature

Notation

a, b	constants for ethanol formation, growth associated and non-growth associated, respectively
α_t	available surface area for cellulose
(B), (C), (E), (G), (X)	concentrations of cellobiose, cellulose, ethanol, glucose and cell mass, respectively
$(E_1)_t$	total concentration of cellulase in the solution
(E_2)	concentration of β-glucosidase in the solution
k_d	specific rate of cell death
k_1, k_2	specific rates of cellulose and cellobiose hydrolysis, respectively
K_I	constant of cell growth inhibition by glucose
K_m	Michaelis constant of β-glucosidase for cellobiose
K_3, K_4	Monod constants of glucose for cell growth and ethanol synthesis, respectively
K_{1B}, K_{2B}	Inhibition constants of cellulase and β-glucosidase by cellobiose, respectively
t	time
K_{1G}, K_{2G}	inhibition constants of cellulase and β-glucosidase by glucose, respectively
K_{1E}, K_{2E}, K_{3E}	Inhibition constants of cellulase, β-glucosidase and cell growth by ethanol, respectively
m	specific rate of substrate consumption for maintenance requirements
μ_m	maximal specific growth rate
$Y_{X/G}$	average yield coefficient of cell mass on substrate (glucose)

cellobiose (dimer hexose sugar units), which are later split into monomeric hexose sugar units by cellobiase (or β-glucosidase). SSF process reduces the inhibitory effect of substrate (sugar) concentration on enzymes and also the probability of contamination by undesired invasive microorganisms [4]. These features increase yield as well as kinetics of the saccharification as well as fermentation as compared to the conventional two-step process.

More recently, another technique of ultrasound irradiation or sonication of the fermentation broth for intensification of bioethanol fermentation has been attempted. Ultrasound is a well known technique for intensification of diverse physical and chemical processes [5–10]. Ultrasound manifests its effect on the reaction system through phenomenon of cavitation, which is nucleation, growth, oscillation and implosive transient collapse of tiny gas or vapor bubbles, which is driven by pressure variation generated in the medium during passage of ultrasound wave. Both ultrasound and cavitation render several physical and chemical effects on the reactions system, which are beneficial in enhancing the kinetics of the system. The most peculiar feature of energy introduction into the medium via ultrasound and cavitation is that implosive collapse of cavitation bubbles creates intense energy concentration on an extremely small spatial and temporal scale. The main physical effect of ultrasound and cavitation is generation of intense micro-turbulence in the medium that gives very effective micromixing, which eliminates mass transfer limitations. The chemical effect of transient cavitation is generation of highly reactive radicals and other smaller species through dissociation of vapor entrapped in the bubble at the moment of transient collapse. Literature on application of ultrasound during bioethanol synthesis through SSF process is rather limited. Wood et al. [11] have reported bioethanol production using ultrasound (36 kHz, 150 W) assisted SSF process. The substrate used was waste paper and microbial strain of *Klebsiella oxytoca* was employed. Bioethanol yield was found to increase by 20% with sonication. Ofori-Boateng and Lee [12] have investigated bioethanol production using SSF process from oil palm fronds as substrate and *Saccharomyces cerevisiae* as the microbial strain. With ultrasound of 40 kHz frequency and 200 W intensity, 4-fold increment in bioethanol yield was observed within 5 h.

In order to effectively utilize the potential of ultrasound on intensification of the SSF process for bioethanol production, it is essential to understand the basic underlying physical mechanism. This would essentially mean identifying the links between physics of ultrasound and cavitation and the biochemistry of fermentation. In this paper, we have addressed this important issue with the approach of coupling experimental results to the fermentation model of [13], which comprises of 5 ordinary differential equations, viz. one each for cellulose, cellobiose, glucose, microbial cell concentration and ethanol. This model takes into account the essential physiology of the SSF process. A major practical limitation of implementation of this model is difficulty in monitoring of the concentration of cellulose (which occurs in solid phase) and also the unstable intermediate of hexose-dimer cellobiose (which is rapidly decomposed into monomeric glucose) during fermentation [14]. Despite this limitation, fitting of the experimental data of microbial cell concentration and ethanol to their respective differential model equations reveals important mechanistic account of the influence of ultrasound on the SSF process. In our experiments, we have used waste biomass of *Parthenium hysterophorus* as substrate with *S. cerevisiae* as the microbial strain.

2. Materials, methods and mathematical model

2.1. Chemicals and reagents

All components of fermentation medium were procured from HiMedia Pvt. Ltd., India. Glucose (99.5% purity, standard for HPLC and reducing sugar estimation) was procured from Sigma Aldrich, USA. Ethanol (99.5% purity) was procured from Tedia Chemicals, USA. All other chemicals were procured from Fischer Scientific, India.

2.2. P. hysterophorus biomass

P. hysterophorus biomass was collected from the campus of our institute. Biomass was chopped (~5 cm), washed with water, dried at 60 ± 3 °C for 24 h and ground to a particle size of ~1 mm. Powdered biomass was pretreated with 1% (v/v) H_2SO_4 + 30 min autoclaving [15], and the solid residue was further delignified by ultrasound assisted alkaline treatment [16].

2.3. Source of enzymes

Carboxymethylcellulase (CMCase) (1.0 U/mg, 1.7 mg/mL) was produced from *Bacillus amyloliquefaciens* SS35 [17–19] and β-glucosidase (250 U/mL) (Novozyme 188) was procured from Sigma Aldrich, USA.