Production of bioethanol from lignocellulosic banana peduncle waste using *Kluyveromyces marxianus*

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Abstract

Aim: Bioethanol production from lignocellulosic material (banana peduncle) as a substrate using microorganism *Kluyveromyces marxianus* MTCC 1389 was carried out in this study.

Methodology: The substrate was prepared by mechanical grinding, followed by dilute acidic treatment using H₂SO₄ with various concentrations such as 1, 2 and 3%, respectively. Temperature was maintained at 150°C for breaking the glycosidic linkage and converting into monomer. The chemical composition and concentration of various constituents in the raw material was characterized. Fermentation was carried out at various temperatures (25, 30, 35 and 40°C) and pH (4.0, 4.5, 5.0 and 5.5). Batch fermentation kinetics of bioethanol production was studied.

Results: The maximum biomass and ethanol yield was achieved at 40°C and pH 4.5 with specific growth rate (μ) 0.021 hr⁻¹, biomass yield ($Y_{x/s}$) 14.7 g l⁻¹ and bioethanol yield ($Y_{p/s}$) 21.89 g l⁻¹.

Interpretation: Lignocellulosic material obtained from waste banana peduncle can be utilized for bioethanol production.

Key words : Acid hydrolysis, Banana peduncle, Bioethanol, *Kluyveromyces marxianus*, Lignocellulose

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Introduction

The use of renewable resources for biofuel production is growing rapidly due to the production of plenty of agricultural wastes, advancing in civilization and draining of fossil fuel. This new technology has increased the value of agricultural products, instigating into investment, enhancing economic opportunity and also reduces the environmental impact (Elsa *et al.*, 2015a; Elsa *et al.*, 2016). Ethanol produced from waste plant biomass is an eco-friendly approach, reduces the use of fossil fuel, advances the farm economy with clean environment. Waste lignocellulosic materials obtained from energy crops of second generation, like agricultural residues and wood are rich source of renewable energy (Lin and Tanaka, 2006; Sivarathna kumar *et al.*, 2014; Elsa *et al.*, 2014; Baskar *et al.*, 2016; Sivarathnakumar *et al.*, 2017).

Kluyveromyces marxianus IMB strain in simultaneous saccharification and fermentation shows favorable yield between 40 to 50°C (Faga *et al.*, 2010). *K. marxianus* Y179, yields maximum ethanol of 98 g l⁻¹ at 30°C (Gao *et al.*, 2015). This thermophilic and ethanogenic specie cab be used in simultaneous saccharification and fermentation for ethanol production for limiting the cost of overall process (Choudhary *et al.*, 2016; Murata *et al.*, 2015; Raja Sathendra *et al.*, 2019). Thus in the present study, high thermo tolerance *K. marxianus* species was used for the production of bioethanol.

Materials and Methods

Substrate: The substrate (banana peduncle) used for bioethanol production was collected from the local market, Tiruvannamalai, Taminadu, India. It was washed with distilled water to remove dust and contaminants. The sample was cut into small pieces to increase the surface area and shade dried to remove moisture and stored in a tight container for further use.

Characterization of substrate: The substrate was made into a powder by grinding. The chemical composition of the substrate was analyzed using chromatographic analysis.

Hydrolysis of substrate with dilute acid: A known amount of substrate for ethanol production was taken in three beakers containing 1, 2 and 3% HCl, maintained at 150°C. Pretreatment using dilute acid increase the reducing sugar yield from lignocellulosic substrate. Dilute H₂SO₄ between 0.2 and 2.5% at 130 to 210°C enhanced the cellulose hydrolysis process (Balat *et al.*, 2008). Disadvantage of acid hydrolysis is the release of inhibitors such as furfural and 5-hydroxymethylfurfural (HMF) which will inhibit the growth of the microorganism during fermentation. Saccharification of wheat straw yields 74% reducing sugar when 0.75%(v/v) of H₂SO₄ was used at 121°C for 1 h (Saha *et al.*, 2005).

Inoculum development: Microorganism, *K. marxianus* MTCC 1389 was cultivated in Malt Yeast Agar, which contained 3 g l⁻¹ malt

extract, 3 g l⁻¹ yeast extract, 5 g l⁻¹ peptone, 10 g l⁻¹ glucose, 20 g l⁻¹ agar, 1 l distilled water at pH 4.5 and temperature 35 °C.

Detoxification of hydrolysate: Dilute acid hydrolyse of hemicelluloses and plant component was used to release the reducing and non-reducing sugar along with certain chemicals like furfural and 5-hydro-di-methyl furfural. These chemicals have inhibitory effect on microorganism during fermentation. Adding lime in conjunction with high pH and temperature is a promising detoxification technique for dilute H₂SO₄ pretreated hydrolysate of lignocellulosic substrate for the removal of inhibitory volatile compound such as furfural and HMF from the hydrolysate (Chandel *et al.*, 2007; Chandel *et al.*, 2011).

Optimization of temperature and initial pH for bioethanol production: The hydrolyzed substrate was adjusted with pH and used for ethanol production. The ethanol production of *K. marxianus* MTCC 1389 was carried out in 500 ml Erlenmeyer flask containing 100 ml concentrated pretreated substrate (banana peduncle) supplemented with 3 g l⁻¹ malt extract, 3 g l⁻¹ yeast extract, 5 g l⁻¹ peptone. The temperature varied from 30, 35, 40 and 45°C at initial pH of 4.5. The pH varied from 4.0, 4.5, 5.0 and 5.5 at initial temperature of 40°C.

Estimation of glucose: The concentration of reducing sugar in hydrolysate was estimated using standard DNS method (Tasun *et al.*, 1970).

Estimation of ethanol and biomass: Ethanol produced was estimated by potassium dichromate method (William and Reese, 1950) and biomass was estimated by dry cell weight method.

Kinetic modeling: Kinetics model express the stoichiometric relation between product formation and substrate utilization (monitoring and predicting the fermentation process of glucose). In batch fermentation, the kinetic model provide information to predict the rate of biomass and product formed. The product formation kinetics was studied using Leudeking-Piret model as given in eq. 1. Leudeking-Piret model consider the relationship between biomass growth to the rate of product formed (Doran, 2003; Zafar *et al.*, 2005).

$$\frac{dp}{dt} = \alpha \frac{dx}{dt} + \beta x$$

The graph was plotted between product concentration and time. The slope (dp/dt) at different point was determined. The graph was plotted between (1/x)(dp/dt) and (1/dx)(dx/dt) and from this intercept α and slope β were determined.

Under optimum growth conditions and in the absence of inhibitory effects of substrate and product, the rate of formation of biomass increases exponentially (Doran, 2003; Ariyanti and Hadiyanto, 2013). It is represented by Monod kinetic model given in eq. (2-4).

$$\frac{dp}{dt} = \mu x \quad (2)$$

$$\frac{1}{v} = \frac{K_m + [s]}{V_m [s]} = \frac{K_m}{V_m [s]} + \frac{s}{V_m [s]} \quad (3)$$

$$\frac{1}{v} = \frac{K_m}{V_m} \cdot \frac{1}{s} + \frac{1}{V_m} \quad (4)$$

The specific growth rate (μ) $= (1/x)(dp/dt)$. The graph was plotted between $1/\mu$ with $1/S$ from this graph the intercept $1/\mu_{\max}$ and slope $K_s/\mu_{\max}$ was calculated.

Results and Discussion

The GC-MS analysis of substrate showed the presence of several compounds. The concentration of glucose was 0.17% with generic name of 3-deoxy-d-mannonic acid with retention time of 10.7 min, area of 13395 (0.03%) and peak height of 13310 as shown in Fig. 1.

The maximum concentration of reducing sugar was obtained with 3% H_2SO_4 as compared to other hydrolysate broth. This hydrolysate was utilized for fermentation using *K. marxianus*. The concentration of ethanol at different temperature

and pH are shown in Fig. 3 and 4, respectively. The increase in bioethanol was observed with increase in temperature from 30 to 40°C (Fig. 3). The production of bioethanol concentration decreased with further increase in temperature from 40 to 45°C. Ethanol production was considerably low at 30 and 35°C due to low rate of microbial growth at low temperature. The maximum bioethanol (21.87 g l⁻¹) was obtained at 40°C in 96 hr. The bioethanol production increased at initial pH from 4 to 4.5, then decreased with further increase in initial pH from 4.5 to 5 (Fig. 4). The maximum bioethanol (21.89 g l⁻¹) was obtained at initial pH of 4.5 at 40°C in 96 hr. The acidic pH was better for the growth of *K. marxianus*, which might be the reason for maximum bioethanol yield at pH 4.5 (Elsa et al., 2015b; Sivarathnakumar et al., 2017).

The lag phase was observed during fermentation between 0 to 12 hr for cell to adapt to the fermentation medium (Doran, 2003). The concentration of biomass started increasing after 12 hr of fermentation and reached maximum biomass concentration (14.7 g l⁻¹) in 96 hr. Utilization of reducing sugar took place during fermentation after 12 hr and it decreased faster after 24 hr. Ethanol production increased after 12 hr due to the increased consumption of reducing sugar and high biomass growth rate (Fig. 5). The concentration of reducing sugar reduced from 40 g l⁻¹ to 9 g l⁻¹ at 96 hr. The maximum biomass and bioethanol concentration obtained was 14.7 g l⁻¹ and 21.89 g l⁻¹ in 96 hr. The hydrolysate of 3% H_2SO_4 pretreated lignocellulosic

Fig. 1 : Chemical composition of banana peduncle in raw sample.

Fig. 2 : Concentration of reducing sugar in pretreated substrate hydrolysate.

Fig. 3 : Effect of temperature on bioethanol production (Initial pH fixed at 4.5)

Fig. 4 : Effect of different pH on bioethanol production (Temperature fixed at 40°C)

Fig. 5 : Effect of concentration of biomass, substrate and ethanol with respect to time during fermentation (Temperature fixed at 40°C; Initial pH fixed at 4.5)

material produced maximum ethanol concentration of 21.89 g l^{-1} in 96 hr under optimum temperature of 40°C and initial pH of 4.5.

The batch fermentation kinetics of *K. marxianus* was studied by using experimental results at 40°C and pH 4.5. The maximum ethanol yield of 21.89 g l^{-1} and biomass 14.7 g l^{-1} was obtained (Fig.5). The Peudeking-Piret model is growth associated process, where the specific growth rate of product is directly proportional to the specific growth rate of biomass. This significantly indicates the growth associated constant (α) as 0.015 value and non-growth associated constant (β) as 0.043. The α value was 1.52 and β was $0.11 \text{ g l}^{-1} \text{ h}^{-1}$ (Ariyanti and Hadiyanto, 2013). The substrate saturation constant K_s was 1.285 g l^{-1} and the maximum specific growth rate $\mu_{\max}$ was 0.021 hr^{-1} as calculated by Monod model. The biomass yield coefficient ($Y_{x/s}$) was 14.7 g l^{-1} . The value of K_s was 20 g l^{-1} and $\mu_{\max}$ was 0.55 hr^{-1} and $Y_{x/s}$ was 0.25 g l^{-1} (Longhi et al., 2004). The K_s value was 16.088 g l^{-1} and the value of $\mu_{\max}$ was 0.401 hr^{-1} and $Y_{x/s}$ was 0.219 g l^{-1} (Zafar et

al., 2005). The value of K_s was 10.52 g l^{-1} and value of $\mu_{\max}$ was 0.32 hr^{-1} at 34°C (Ariyanti and Hadiyanto, 2013). This may be due to different substrate, operating conditions or modeling strategy.

The results of batch fermentation reported in previous studies are as follows: The specific growth rate (μ) was 0.138 h^{-1} , $Y_{x/s}$ 0.37 g g^{-1} and $Y_{p/s}$ 0.085 g g^{-1} at temperature 30°C (Lukondeh et al., 2005). The specific growth rate μ was 0.0084 h^{-1} , $Y_{x/s}$ 0.16 g g^{-1} and $Y_{p/s}$ 0.054 g g^{-1} at temperature 26°C (Ozmihci and danKargi, 2007). The specific growth rate was 0.186 h^{-1} , $Y_{x/s}$ 0.32 g l^{-1} and $Y_{p/s}$ 0.21 g g^{-1} at 30°C (Hadiyanto et al., 2014). In the present study, the specific growth rate μ was (0.021 hr^{-1}) , $Y_{x/s}$ (0.58 g g^{-1}) and $Y_{p/s}$ (21.89 g l^{-1}) at 40°C . This shows that *Kluveromyces marxianus* is an efficient thermo tolerance species and can produce ethanol at elevated temperature. The regression co-efficient value (R^2) of 0.965 indicates, product formation kinetic model effectively described the actual changes of bioethanol production during fermentation. The arrangement of component in the plant

biomass which in turn interacts with hemicelluloses and lignin make it hard for utilization, therefore, at primary step the size of the plant biomass is reduced to 5-25 mm through chopping and grinding (Chundawat *et al.*, 2011; Kumar *et al.*, 2009). Lignocellulosic materials are highly complex polymer that contain cellulose, hemicelluloses and lignin. Hemicelluloses are heterogeneous polysaccharide with β -(1-4)-linked strength of character having hexose (C_6) sugar (Scheller and Ulvskov, 2010). Hemicelluloses are short and highly branched is a heteropolymer. Lignin is hydrophobic in nature and is tightly bound to these two polymers and has protective function (Peiji *et al.*, 1977).

The acid pretreatment of lignocellulosic material make the hemicelluloses soluble, and thus cellulose become more accessible (Hendriks and Zeeman, 2009; Sivarathna Kumar *et al.*, 2016a). Perhaps, pretreatment and hydrolysis is considered to be the most expensive and important steps in the processing of lignocellulosic materials for bioethanol production (Scheller and Ulvskov, 2010). Dilute acid pretreatment is economically competitive, that requires expensive acid-resistant stainless steel unit for pretreatment due to corrosion problems (Kim and Holtzapple, 2005; Sivarathna Kumar *et al.*, 2016b).

Thus, the results of this study indicates *K. marxianus* as a highly promising candidate for bioethanol production.

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