Biomass and bio-ethanol production from date extract

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Abstract

In recent years, increasing attention has been pointing to produce bioethanol product using biological method. For this reason our study focused to fermentation process which was an approach to waste date products valorization through biomass production with the yeast *Saccharomyces cerevisiae.* Bioethanol could be easily produced by alcoholic fermentation of yeast fermentable sugars. The result showed that date waste can constitute an industrial substrate for the production of biomass "yeast" $Y_{X/S} = 0.45$ and bioethanol $Y_{P/S} = 0.51$.

1. Introduction

Recently, increased interest in fuel from biomass in the worldwide has emerged each time petroleum derived gasoline registered well publicized spikes in price. Industrial research efforts have become more focused on low-cost large-scale processes for lignocellulosic feed stocks originating mainly from agricultural and forest residues along with herbaceous materials and municipal wastes [1]. Several researchers working on efficiencies of Biomass and bio-ethanol production. Jaime Barros-Rios*et al*. used the hydrothermally pretreated stover biomass from maize genotypes by bioethanol production [2]. Lee et al., have realized sustainable production of bioethanol using lipid-extracted biomass from Scenedesmus dimorphus [3]. Rokniyahya et al., used natural green olive biomass for fermentation process [4]. Louhichi et al., studies the production of bio-ethanol from three varieties of dates [5].

The date palm *(Phoenix dactylifera)* is a tree of great ecological and economic of oasis desert [6]. In consequence, it is the focus on agriculture and provides the main resource of financial oasis [7]. Their success over a long period can be explained by the nutritional qualities of these particular fruits rich in sugars [8], that most of the carbohydrates in dates are in the form of reduced sugars, mainly fructose and glucose [9].

The waste recycling process of date fruit is considered the most important biological process within the environmental system that aims to keep the environmental balance. Biological reactions are one of the safest and most successful methods, in which microorganisms such us *Saccharomyces cerevisiae* and *Zymomonas mobilis*can play an important biological role and restore the balance within the environmental system, in addition to their success in transforming date palm wastes to products of an economic return [10].

Bioéthanol is also an important renewable and sustainable alternative clean fuel source [11-12]. Nowadays, the world fuel bioethanol production exceeds 20.000 millions of gallons per year [13-14]. Several review articles describing the various methods for bio-ethanol production, studies include enzymatic fermentation, simultaneous saccharification and fermentation, enzymatic hydrolysis, process modelling, supply chain simulations [15-16]. Selective fermentation is an efficient process for large scale production of bioethanol. In fermentation process, *Saccharomyces cerevisiae* yeast can be used to produce bioethanol [17].

The present work focuses on the most interested biomass and bio-ethanol production from date's extract by fermentation using *S. cerevisiae* strain, and the assessment of kinetics for subtract conservation converted into bio-ethanol.

2. Materials and methods

2.1. Preparation of the fermentation broth

2.5 liters of distilled water is added to 1 kg of washed, pitted and crushed dates. The juice is heated at 85 °C for 45 minutes under continuous stirring. Total sugars concentration was adjusted to 200g/l by dilution and filtered. After that, mineral solution (Urea 0.3g, KH_2PO_4 0.05g, K_2HPO_4 0.05g, $MgSO_4$, $7H_2O$ 0.05g, $FeSO_47H_2O$. 0.001g per 100ml) was added and the pH was adjusted to 4 using 1 M Tartaric acid solution before autoclaving.

2.2. Fermentation

Fermentation tests were performed using the selected yeast *Saccharomyces cerevisiae*s trains isolated from date's samples and identified according to krejer van rij 1984 keys [18]. Yeast fermentation tests were conducted in Erlenmeyer flasks shakedin an orbital system 60 rev/min. Yeast biomass produced by overnight culture was used as inoculum to start up fermentation 1%.The incubation time was 3 days under semi-anaerobic conditions. The samples were withdrawn at appropriate time intervals for the analysis.

2.3. Estimation of biomass concentration

The biomass was determined by the dry weight method. The cells were separated by centrifuging at 3500 rpm for 20 min. the pelut was consecutively twice washed with distilled water and centrifuged. The cells were dried at 100°C until the constancy of biomass. Dilutions of the culture were also made and the absorbance was measured. The calibration curve correlating absorbance and dry weight gave a straight line [19].

Active culture for inoculation were obtained in 500 ml Erlenmeyer flasks with 100 ml of malt extract broth and incubated at 30 °C, 100 rpm for 24 h. The preculture was centrifuged at 10000× g for 10 min and the supernatant was discarded. The cells were re-suspended in sterile physiological water and were adjusted to obtain a concentration of about 1×10^7 cells m/l estimated by using a hematocytometer (Counting chamber), the temperature was also maintained at different range as per experiment.

2.4. Estimation of sugar concentration

A 5 ml aliquot of fermented sample was centrifuged at 10000 rpm for 10 min. The supernatant solution was used to determine the Sugars concentration by DNS method [20].

2.5. Model development

Kinetic model, among the numerous models developed, the majority models consist of mathematical equations describing the phenomena occurring during wine fermentation. The main advantage of this type of model is that they account for biological phenomena. The model parameters with some biological significance can be obtained, but their structures may be strongly nonlinear, complex and difficult to verify and validate [21-22].

In general, the fermentation kinetic model can be subdivided into a growth model, a substrate model, and a product model. There are three different equations derived to describe the kinetic behavior of the concentration of yeast cells, the sugars (glucose), and ethanol in this study.

For cell concentration, X, the logistic model was derived as follows (Eq.1):

$$\frac{dX}{dt} = \mu_m X \left(1 - \frac{X}{X_m}\right) \qquad (Eq.1)$$

Where μ_m is the maximum specific growth rate with respect to the fermentation conditions, as the form of the Monod relationship. With the following boundary conditions:

$$t = 0 \quad ; \quad X = X_0 \quad ; \quad S = S_0 \quad , \quad P = 0$$

The equation of ethanol production rate was modified as Eq.2:

$$\frac{dP}{dt} = Y_{p/x} \frac{dX}{d(t - \Delta t)} \qquad (Eq.2)$$

3. Result and discussion

3.1. Growth kinetics of yeast Saccharomyces cerevisiae with different concentration of sugar

To determine the optimum concentration of sugar required for the growth of the yeast *Saccharomyces cerevisiae*, we conducted a series of kinetics fermentation. Figure 1 illustrates the growth of the biomass (x) at different concentrations of reducing sugars. The concentrations of reducing sugar tested are respectively 2, 4, 6, 8 and 10 g/l. This range was established to mark the passage of oxidative in fermentative metabolism. The result showed that 6 g/L of reducing sugar present a very good concentration for biomass production.
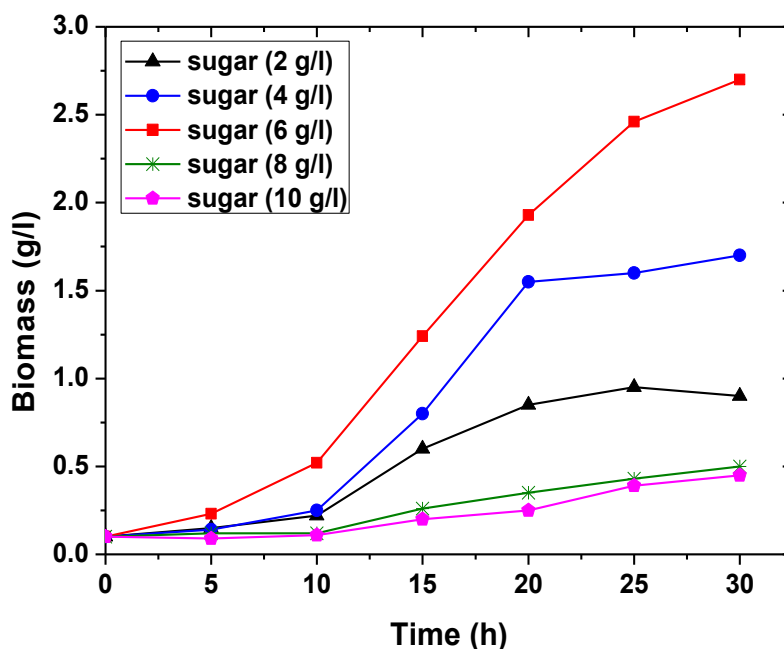

Figure 1: Experimental data and kinetic model predictions for *Saccharomyces cerevisiae strain* growth, using reducing sugar at different concentration

These results are in agreement with the literature and industrial practices well trying to benefit from the conversion of biomass substrate avoiding inhibition by excess substrate and accumulation of toxic substances [23]. Which indicates that the fatty acids, especially octanoic and decanoic acid, formed by the yeast become toxic to the latter, and that the effect of alcohol and glucose in the medium becomes inhibitor [24].

The yeast used sequentially fermentable sugars present in the date syrup, sucrose is hydrolyzed into glucose and fructose by enzyme located in the outer surface of yeast. These sugars are consumed simultaneously converted into biomass, ethanol and CO_2 [25].

3.2. Growth Rate of biomass formation for Saccharomyces cerevisiae strain

Figure 2 shows the growth rate ($\mu_m = r_x / X$) as a function of the substrate concentration S (reducing sugars) in the culture medium based scrap dates. It shows an increase of 2 to 6 g/l of sugar. The yeast maximum specific growth rate (μm) is highest $0.23h^{-1}$ at 6 g/l. above this concentration, there was a significant reduction in the growth rate, which announces the passage of oxidative metabolism to a fermentative metabolism. This can be explained by the effect of high substrate concentrations on the respiratory chain (Crabtree effect), whose must be considered as the repression of an energy-producing system, respiration, by another energy-producing system, fermentation. Thus, when respiration occurs simultaneously with aerobic fermentation, as is the case with the normal strain of *S .cerevisiae*growing on galactose,despite aeration which exceeds the metabolic demands of the cells (8-10 g/l).

Growth rates found in our study are lower compared to those studied in the bibliography on industrial culture media used; this fact is due to the lack of nitrogen, phosphate and other growth factors in our environment culture. Thus, several studies indicate that nitrogen supply to crops improves the yields of biomass and ensures sustained absorption of sugars [26]. Hariri *et al* notes that the *Saccharomyces cerevisiae* growth rate grown on favorable medium between 0.3 and 0.47 h^{-1} [25].
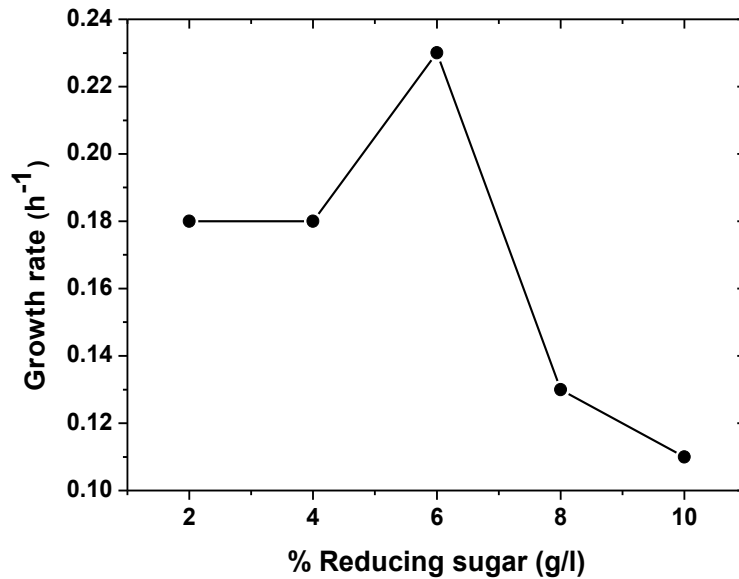
Figure 2: Growth rate of yeast *Saccharomyces cerevisiae* with different concentration of sugar

3.3. *Weight biomass yield compared to diluted substrate at different concentrations.*

The weight yield ($Y_{X/S} = x / s$) of the biomass (x) with respect to the substrate (s) was evaluated to determine the concentration limit promoting biomass production under oxidative conditions and not fermentative (Crabtree effect). Figure 3 presents the weight yields expressed as dry weight of biomass ($Y_{X/S}$). It notes that although the biomass x is very high at 8g/l compared to 6g/l of reducing sugar; there is a significant reduction in the weight yield of $Y_{X/S}$. This was confirmed by the samples distillation, which confirms the absence of ethanol in the culture media (2, 4 and 6 g/L of reducing sugar) and the presence of ethanol in concentrations ranging from 8 and 10 g/l of the substrate, due to blockage of the breathing by excess substrate.
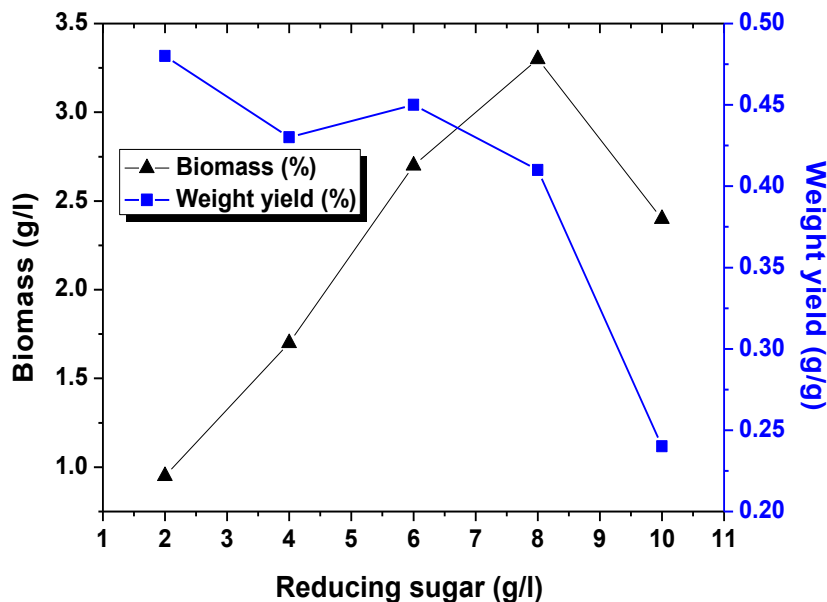

Figure 3: weight yield of biomass (X) as a function of sugar concentration in date syrup

3.4. *Optimization of operational parameters for Ethanol Production*

The growth kinetics of biomass (X), substrate consumption (S) and ethanol formation (P) were evaluated in terms of samples (every 10 hours). Figure4 shows the mass production of ethanol from the beginning of the fermentation cycle. This is due mainly to the pre-overnight culture that adapted inoculum in fermentation

medium. Based on the result obtained from the kinetic study, we see that there is a drop in the substrate inversely proportional to the increase in bioethanol. The increase in biomass is very low because of the fermentation trend exerted by the high sugar concentration that inhibits breathing (Crabtree effect) and consequently, there is a sporadic flow of glycolysis, which attempts to provide the energy required ATP and recycled reducing power to ensure continuity of alcoholic fermentation [27].
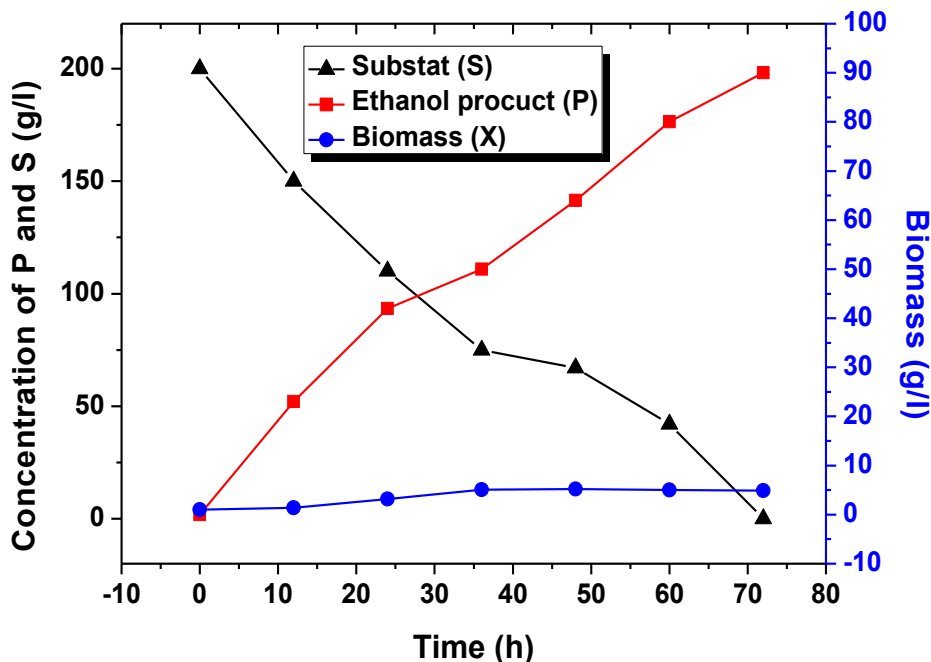

Figure 4: Ethanol, biomass production and sugar consummation

The maintaining cells for fermentation process were evaluated. The results showed that 13.4 g/l present fraction of substrat destined for maintaining cells, the Table 1 present Kinetic parameters estimated from the experimental data on fermentation process.

Table 1: Kinetic parameters for biomass and bioethanol production

Kinetic parameters	Value
weight yield (Y=P/S) for Bioethanol production (g/g)	0.51
weight yield (Y=X/S) for Biomass production (g/g)	0.5
Fraction of sugar transformed on Bioethanol (S_p) (%)	88
Fraction of sugar transformed on biomass (S_x) (%)	5.3
Fraction of sugar use for maintaining cells (S_m) (%)	6.7

Conclusion

This kinetic study, we retain the majority of the substrate is converted into bioethanol (88%). This is for the use of scrap dates as fermentation substrate. The fraction for biomass and cell service is low, which justifies the choice of strategic sustainable recovery of scrap dates by fermentation engineering. The produced biomass could be valued as unicellular organism proteins (POU) and this may contribute to the increase of the profit margin of the proposed method.

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