

Research article

Prospective production of fructose and single cell protein from date palm waste

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ARTICLE INFO

Article history:

Received 26 April 2020

Accepted 14 September 2020

Available online 18 September 2020

Keywords:

Aerobic

Date syrup

Fructose

Gompertz model

Palm date

Palm waste

pH

Saccharomyces cerevisiae

Selective fermentation

Single cell protein

Syrups

ABSTRACT

Background: Fructose and single cell protein are important products for the food market. Abundant amounts of low-grade dates worldwide are annually wasted. In this study, highly concentrated fructose syrups and single cell protein were obtained through selective fermentation of date extracts by *Saccharomyces cerevisiae*.

Results: The effect of air flow (0.1, 0.5, 0.75, 1, 1.25 and 1.5 vvm) and pH (4.5, 4.8, 5, 5.3 and 5.6) was investigated. Higher air flow led to lower fructose yield. The optimum cell mass production of 10 g/L was achieved at air flow of 1.25 vvm with the fructose yield of 91%. Similar cell mass production was obtained in the range pH of 5.0–5.6, while less cell mass was obtained at pH less than 5. Controlling the pH at 4.5, 5.0 and 5.3 failed to improve the production of cell mass which were 5.6, 5.9 and 5.4 g/L respectively; however, better fructose yield was obtained.

Conclusions: Extension of the modified Gompertz enabled excellent predictions of the cell mass, fructose production and fructose fraction. The proposed model was also successfully validated against data from literatures. Thus, the model will be useful for wide application of biological processes.

How to cite: Putra MD, Abasaheed AE, Al-Zahrani SM. Prospective production of fructose and single cell protein from date palm waste. Electron J Biotechnol 2020;48. <https://doi.org/10.1016/j.ejbt.2020.09.007>.

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1. Introduction

Due to its high sweetness (almost twice that of glucose), fructose is one of the important sweeteners that has been utilized in industrial beverages and foods, e.g., drinks, doughnuts, chocolate, cereal, ice cream, sweetened yogurt or candy [1]. Compared to other sweeteners, fructose provides advantageous characteristics including higher solubility thus difficult to be crystallized from its aqueous solution [2], and easier to absorb moisture and slower to release it to the environment [3]. High fructose corn syrup (HFCS) represents about 40% of all added sweeteners to food and beverages in the US diet [4].

About half of the production of date is wasted and thus unutilized [5,6]. The worldwide production was about 8.5 million tons in 2016 [7]. Almost half of the sugar in dates is fructose [8]; and over 75% of dates is reduced sugars on dry basis [9]. Thus, date is a prospective raw material for the production of fructose and single cell protein.

The commercial enzymatic process provides only 42% fructose in the fructose-glucose mixture (HFCS-42). The production of 90% fructose syrup is industrially carried out via the high-cost continuous multistage

chromatography [10,11]. To obtain high fructose syrup (90%), processes such as membrane system separation [12] and ionic liquid solvent extraction [13] should be economically evaluated since the materials used are expensive. Furthermore, a selective fermentation utilizing microorganisms was shown to be a very promising alternative process for the production of fructose and ethanol [14]. However, ethanol purification to high value (>95%) is also complex and costly [15,16]. Thus, to alleviate this concern, simultaneous production of fructose and single cell protein (replacing ethanol) should be considered.

Single cell protein (SCP) is widely used as animal and human fodder, biocatalyst in medical industry, and aroma and vitamin carrier [17]. With increased world human population, the availability of a simple food supplement such as SCP is highly required [18]. *Saccharomyces cerevisiae* yeast has been used as a protein source for humans and animals to produce vaccines, growth hormone, and renin [19]. Yeast is a potential source of SCP due to their flocculation abilities and their relatively bigger sizes [20]. Yeast also provides many advantages such as high protein content, rapid growth rate, no pathogenicity, fast digestibility and palatability [21,22].

The presence of oxygen in aerobic fermentation inhibits the pathway to ethanol production and increases the growth rate of microorganism [23]; the optimum airflow in batch systems also depends on the substrate type and microorganism used. The

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Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

concentration of ethanol was suppressed when increasing oxygen flow up to 10% [24]. The effect of pH on cell growth is also very essential; the pH could affect the growth and nutrients added to the system [25]. Furthermore, the pH limits for cell growth for *S. cerevisiae* are between 2.4 to 8.6 with the optimum growth at range of 4–5 [26].

In this study, the performance of *S. cerevisiae* in the aerobic selective fermentation of date extracts for simultaneous production of fructose and single cell protein was investigated. The effect of air flow rate and pH were evaluated. An extended model based on the modified Gompertz equation was also introduced for prediction cell mass, fructose and fructose fraction profiles. The model is very useful to apply in many fermentation processes and also for further process development.

2. Materials and methods

2.1. Preparation of microorganism and media

Saccharomyces cerevisiae ATCC 36858 (Manassas, VA, USA) was used in this investigation. The reviving of the yeast was based on ATCC procedure. The incubation of the yeast was conducted in agar slant at 30°C for 5 d in an incubator (Jeio-tech Model ON-12 G, Seoul, South Korea). Liquid medium (LM) containing 10 g dextrose, 10 g yeast extract, 3.5 g peptone, 2 g KH₂PO₄, 1 g MgSO₄·7H₂O, 1 g (NH₄)₂SO₄, 1 g FeSO₄·7H₂O and de-ionized water (until 1 L) was prepared for propagation. Before adding the culture to a 500 mL flask containing 100 mL of LM, the flask was sterilized at 121°C for 15 min in an autoclave (Astell AMB230N, Kent, UK). The flask was further shaken in a rotary incubator for propagation (Innova 43 Incubator, CT, USA) at 30°C and 120 rpm for 36 h.

2.2. Preparation of substrate

The extraction of date sugars was conducted using deionized water at 50°C for 2 h with 0.4 weight ratio of dates to water (i.e., 200 g date/500 mL water). The suspended solids and fibers were removed by centrifuging the mixture to obtain the sugars syrup. The syrup was then sterilized at 121°C for 15 min in an autoclave (Astell AMB230N, Kent, UK).

2.3. Fermentation process

The fermentation medium was composed of 90% substrate and 10% culture liquid medium of the yeast. The aerobic fermentation process was conducted in a 500 mL screw capped conical flasks with 100 mL working volume; the flasks were placed in the rotary shaker incubator (Innova 43 Incubator, CT, USA). The fermentation process was conducted at 33°C and agitation speeds 160 rpm. To study the effect of air flow, various air flows, i.e., 0.1, 0.5, 0.75, 1, 1.25 and 1.5 vvm were used at pH of 5.3. The air flow was controlled by flow meters. For the study of pH effect, the pH range of 4.5–5.6 was applied at an air flow 1.25 vvm. The pH of the substrate solutions was adjusted at 4.1, 4.5, 4.75, 5.1 and 5.35; the value of pH changed to be 4.5, 4.8, 5, 5.3 and 5.6 respectively after adding the culture. In case of adjusted pH, a 1 L fermentor was operated with 400 mL working volume. The operating condition applied for fermentor was same with the conical flask, i.e. at 33°C and agitation speeds 160 rpm. In this fermentor, the fermentation process for the adjusted pH of 4.5, 5 and 5.3 were conducted as a comparison to the unadjusted pH process.

2.4. Analysis of samples

One milliliter sample was periodically taken (routinely at the beginning fermentation and at least one time per day afterward up to 7 d) from the fermentation media. The samples were put in a pre-weighed Eppendorf collection tube. A portion of 0.05 mL was injected to NucleoCounter®YC-100TM system (NucleoCounter YC-100, Enfield, CT, USA) to double check the dry weight method. The remaining samples in

the collection tube was then centrifuged at 15,000 rpm for 6 min to separate the cell mass. The solution was withdrawn from the tube and placed in vial for further analysis. The participated cell mass in the tube was washed and re-centrifuged three times to remove any remaining sugar with the cell mass. The cell mass was dried at 80°C over night until the weight was constant. The concentration of the cell mass was determined using the dry weight method by subtracting initial weight of the empty tube from that with dried cell mass. The withdrawn solution (free of cell mass) was used for quantifying the concentrations of the sugars, glycerol, and ethanol by using high performance liquid chromatography (HPLC-Agilent 1200 Infinitely series, Wilmington, DE, USA). The HPLC was equipped with a RID detector and Aminex-® column (150 × 7.8 mm, BIO-RAD®, Foster City, USA). The column temperature was kept at 40°C and sulfuric acid, 1 mM, was utilized for mobile phase with a flow rate of 0.8 mL/min. Duplicate experiments were conducted and the average values of results were presented. Statistical analysis was conducted by using ANOVA.

2.5. Predictive model for cell mass, fructose concentration and fructose fraction

To predict the concentration of cell mass, fructose and fructose fraction, the general Gompertz equation was applied. The general Gompertz equation is described as follows:

$$\gamma_i = \gamma_{i,m} \exp \left[- \exp \left[\frac{r_{i,m} \cdot \exp(1)}{\gamma_{i,m}} (t_L - t) + 1 \right] \right] \quad [1]$$

where *i* denotes product (e.g., cell mass or ethanol), γ_i represents product concentration (g/L), $\gamma_{i,m}$ represents the potentially maximum product concentration (g/L), $r_{i,m}$ is the production rate for maximum product concentration (g/(L·h)) and t_L is the adaptation phase or the time to exponential product concentration (h). The kinetic profiles of aerobic selective fermentation at 33°C and air flow of 1 vvm are discussed below in the section of results and discussion.

3. Results and discussion

3.1. Kinetic profile of aerobic selective fermentation

Fig. 1 presents the profiles of pH, substrates, glycerol, and cell mass concentration. As shown by the figure, the yeast has consumed selectively glucose for growing. At the initial 24 h, small increase of

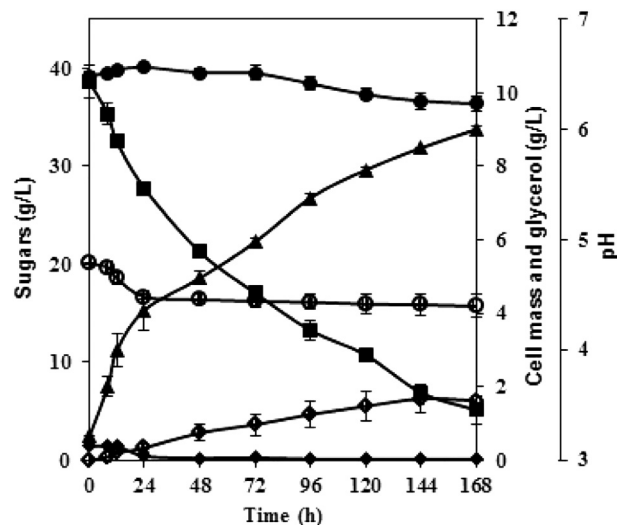


Fig. 1. Kinetic profiles of fructose (●), glucose (■), sucrose (◆), glycerol (◇), cell mass (▲) and pH (○) for aerobic selective fermentation at 33°C and air flow of 1 vvm.

Table 1
Results of aerobic selective fermentation at various air flow.

Flowrate	Final cell mass	Cell mass yield	Fructose yield	Fructose fraction	Sucrose hydrolyzed	Glycerol	Ethanol
vvm	g/L	g/g	%	%	%	g/L	g/L
0	3.65	0.104	101.2	91.0	100.0	0.16	10.92
0.1	6.78	0.163	101.3	91.3	97.7	0.90	8.03
0.5	7.56	0.161	94.9	91.6	92.9	1.03	–
0.75	7.88	0.176	94.3	91.0	93.9	0.81	–
1	9.01	0.226	93.6	90.5	83.5	1.30	–
1.25	10.00	0.223	91.1	90.0	86.8	1.50	–
1.5	9.51	0.204	86.8	90.9	38.1	1.29	–

fructose concentration was observed due to the hydrolysis of sucrose as indicated by the significant drop of sucrose at 24 h (up to 82%); this yeast strain was capable to hydrolyze the sucrose to fructose and glucose as reported in literature [27]. The fructose insignificantly decreased during the fermentation and at 168 h, the fructose yield was 93.6% (i.e., only 6.4% fructose losses). The cell mass increased gradually from 0.6 to 9.0 g/L corresponding to a cell mass yield of 0.226 g/g. It was observed that slope of increase in cell mass during the initial 24 h was about 1.5 times higher than that after 24 h. One fourth of initial glucose was consumed at 24 h due to the inhibition by fructose and fructose fraction in sugar [27]. This is plausible since the fructose fraction increased during the selective fermentation of glucose. The drop in pH during fermentation from 5.3 to 3.9 was attributed to nitrogen assimilation for yeast growth [28]. About 1.3 g/L glycerol concentration was obtained due to the osmoregulation effect of the yeast [29].

3.2. Effect of air flow rate on the performances of aerobic selective fermentation

Table 1 summarizes the results of aerobic selective fermentation of date extract at various air flow rate. Compared to anaerobic process (zero air flow), about twice increase in cell mass when 0.1 vvm air flow was used. Ethanol production was also minimized due to oxidation of the produced pyruvate in the presence of oxygen. It is reported that compared to anaerobic fermentation, the ethanol concentration was found to be optimum at 0.31 vvm using *S. cerevisiae* [30]. It was also noticed that higher glycerol was produced with aeration as associated to osmoregulation effect due to the suppression of ethanol production [29]. Complete oxidation of pyruvate to CO₂ was observed at air flow 0.5 vvm. Increasing air flow led to increase in the final cell mass; however, the optimum cell mass was obtained at 1.25 vvm. The production of cell mass decreased at 1.5 vvm due to inhibition by high oxygen tension. Increase in cell mass yield about 26.7% was obtained with increasing air flow from 0.75 to 1.25 compared to only about 8% increase in the range of 0.1–0.75 vvm. The fructose yield also decreased with increasing air flow; however, the values are still higher than 90% at <1.25 vvm. The

fructose losses were still much lower than those for other microorganisms, e.g., up to 50% for *T. nudum* and *P. pullulans* [31]. It was also noticed that the strain hydrolyzed less sucrose at higher air flow rates.

3.3. Effect of pH on the performances of aerobic selective fermentation

Table 2 presents the results of aerobic selective fermentation at various initial pH. Higher initial un-controlled pH led to higher cell mass production. There were insignificant differences of cell mass production in the pH range of 5.0–5.6; however, the optimum fructose yield was observed at pH 5.3 which was 91.1%. Significant drop of cell mass (about 5 g/L) was observed for pH below 5. It was possible because low pH inhibits cell growth due to organic acid inhibition [32]. For example, in the production of SCP using cheese whey medium, the cell mass obtained at pH of 4.4 was higher than that at pH of 3.5 using *Kluyveromyces fragilis* [33]. For fermentation using *S. cerevisiae*, the yeast could withstand pH in the range of 3.5–7.5; the greatest growth was obtained in the higher pH range of 4.5–5.5 [34]. Furthermore, the fermentation rates are insensitive to values of pH between 3.5 and 6 for non-inhibitory conditions [35].

The effect of pH on the fermentation is difficult to predict [36] and the optimum pH for growth depends on media composition and inhibitory effect [35]. Under controlled pH, there were insignificant differences of cell growth for various microorganism and media [37,38,39]. However, the cell mass production of *L. manihotivorans* was improved at controlled pH [36]. Here, the process with controlled pH either 5.3, or 5, or 4.5 led to drop cell mass production, which were 5.35, 5.85 and 5.60 g/L, respectively. However, the controlled pH plays a positive role in preserving innate characteristic of the yeast strain; thus fructose losses are minimized. It was thus probably that the higher fructose concentration at controlled pH led to inhibition of cell growth.

3.4. Statistical analysis

Significant effect of air flow on the mass cell ($p = 6.27 \times 10^{-6}$) was observed. However, marginally insignificant effect of air flow on the fructose concentration ($p = 0.054$) was obtained. It was possible

Table 2
Results for effect of pH on performance of aerobic selective fermentation.

Initial pH	Final pH	Final cell mass	Cell mass yield	Fructose yield	Fructose fraction	Sucrose hydrolyzed	Glycerol
		g/L	g/g	%	%	%	g/L
4.5	3.5	7.98	0.180	84.8	91.2	0.0	0.69
4.8	3.7	9.21	0.198	83.2	90.3	0.0	1.30
5.0	3.7	10.43	0.238	86.3	90.7	6.0	1.02
5.3	3.9	10.00	0.223	91.1	90.0	86.8	1.50
5.6	4.0	10.34	0.225	87.6	89.8	87.9	1.74
4.5 ^a	4.5	5.60	0.159	98.9	91.8	51.6	1.24
5.0 ^a	5.0	5.85	0.147	98.2	90.9	29.0	1.14
5.3 ^a	5.3	5.35	0.130	98.7	92.9	23.4	0.98

^a Controlled pH.

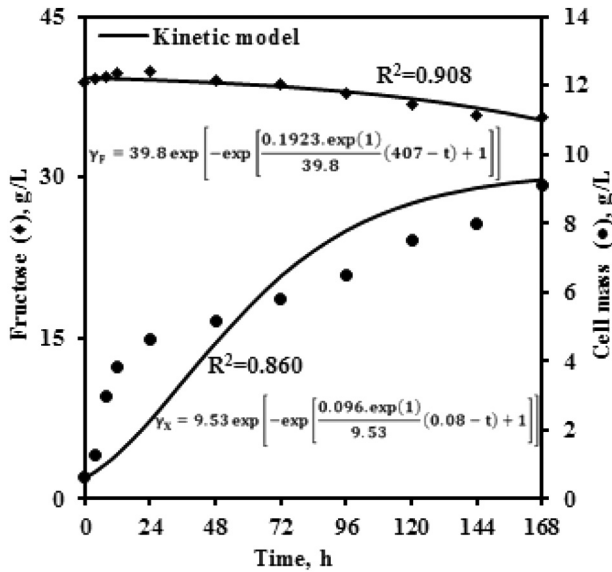


Fig. 2. Kinetic profiles of cell mass and fructose concentration using modified Gompertz model.

because the fructose concentration during the fermentation process remained almost constant due to selective fermentation; *S. cerevisiae* ATCC 36858 does not consume fructose. Similar finding was also observed for the effect of pH on the cell mass with significant effect ($p = 0.000038$). However, insignificant effect of pH on the fructose concentration ($p = 0.269$) was obtained. The reason was in accordance to the effect of air flow on fructose concentration.

3.5. Predictive model for cell mass, fructose concentration and fructose fraction

A model that predicts products formation comprises a step forward for further development of the process [40]. The modified Gompertz model was widely used for the prediction of cell growth and more recently for predictions of various products such as ethanol, hydrogen, surfactant, etc. [41,42,43,44]. As shown in Fig. 2, the modified Gompertz failed to fit the experimental data for fructose and cell mass as indicated by regression coefficient of 0.906 and 0.860, respectively.

For cell mass concentration, due to the inhibition by fructose fraction there were two phases that could not be described by the modified Gompertz model; thus, we have modified the equation as follows:

$$\gamma_X = \gamma_{X,m1} \exp \left[- \exp \left[\frac{r_{X,m1} \cdot \exp(1)}{\gamma_{X,m1}} (t_{L1} - t) + 1 \right] \right] + \gamma_{X,m2} \exp \left[- \exp \left[\frac{r_{X,m2} \cdot \exp(1)}{\gamma_{X,m2}} (t_{L2} - t) + 1 \right] \right] + \gamma_{X0} \quad [2]$$

The first part of [Equation 2] is intended for cell growth without inhibition, while the second part is for cell growth experiencing inhibition by fructose fraction. γ_X is the cell mass production during fermentation (g/L). $\gamma_{X,m1}$ and $\gamma_{X,m2}$ are the cell mass concentration with maximum potential (g/L) for first and second phases, respectively; thus the sum of both represents the potentially maximum total cell mass concentration. $r_{X,m1}$ and $r_{X,m2}$ are the maximum cell mass production rate (g/(L·h)) for first and second phases, respectively. t_{L1} and t_{L2} are the adaptation phases or the time to exponential cell mass concentration (h) for first and second phases, respectively.

Table 3

Predicted parameters of predictive model for cell mass, fructose and fructose fraction.

Products	Parameter	Predicted value	
		First part	Second part
Cell mass	$\gamma_{X,m}$ (g/L)	3.28	5.83
	$r_{X,m}$ (g/(L·h))	0.34	0.044
	t_L	4.9	24.9
	R^2	0.999	
Fructose	$\gamma_{F,m}$ (g/L)	1.653	42.21
	$r_{F,m}$ (g/(L·h))	0.058	-0.092
	t_L	0.001	626.1
	R^2	0.942	
Fructose fraction in sugar	$\eta_{Frcts,m}$ (g/L)	99.8	
	$r_{Frcts,m}$ (g/(L·h))	0.279	
	t_L	1.90	
	R^2	0.983	

For fructose concentration, we also made a provision for sucrose hydrolysis as indicated by small increase in fructose concentration and fructose consumption:

$$\gamma_F = \gamma_{F,m1} \exp \left[- \exp \left[\frac{r_{F,m1} \cdot \exp(1)}{\gamma_{F,m1}} (t_{L1} - t) + 1 \right] \right] + \gamma_{F,m2} \exp \left[- \exp \left[\frac{r_{F,m2} \cdot \exp(1)}{\gamma_{F,m2}} (t_{L2} - t) + 1 \right] \right] \quad [3]$$

where γ_F is fructose concentration during fermentation (g/L). $\gamma_{F,m1}$ and $\gamma_{F,m2}$ are the potential maximum sucrose hydrolysis uptake to fructose concentration (g/L) and the potential maximum fructose concentration, respectively. $r_{F,m1}$ and $r_{F,m2}$ are the maximum fructose production rate (g/(L·h)) at phase of sucrose hydrolysis and the maximum fructose losses rate at phase of fructose consumption. t_{L1} and t_{L2} are the time to exponential fructose concentration (h) at first phase and the maximum time expected for the fructose to be zero (h) at second phase, respectively.

We also expanded the equation to enable the prediction of the fraction of fructose in sugar. The proposed equation was described as follows:

$$\eta_{Frcts} = \eta_{Frcts,0} + (\eta_{Frcts,m} - \eta_{Frcts,0}) \exp \left[- \exp \left[\frac{r_{Frcts,m} \cdot \exp(1)}{(\eta_{Frcts,m} - \eta_{Frcts,0})} (t_L - t) + 1 \right] \right] \quad [4]$$

where η_{Frcts} is the fraction of fructose in syrup (%). $\eta_{Frcts,0}$ is the initial fraction of fructose in syrup (%) (in this case 47.5%). $\eta_{Frcts,m}$ is the potential maximum fraction of fructose in syrup (%), $r_{Frcts,m}$ is the maximum fraction of fructose in syrup (%) and t_L is the time to exponential fraction of fructose (h).

Table 3 presents the parameters of kinetic model for prediction cell mass, fructose and fructose fraction profile during fermentation. The models show excellent fit for all products as shown in Fig. 3 with the regression coefficients of 0.999, 0.942 and 0.983 for cell mass, fructose and fructose fraction, respectively. The model for fructose concentration is very beneficial for prediction of fructose production in many selective fermentation processes such as production of ethanol and fructose on sugarcane, sugar beet molasses, synthetic sucrose, Jerusalem artichoke, inulin etc. [31,45] and production of lactic acid and fructose on sugarcane [46]. The increase in fructose concentration at early 24 h due to the sucrose hydrolysis could be depicted; the concentration then decreased slowly with the expected time to be zero was about 626 h. On the other hand, the model for fructose fraction showed also very good fit to the data; this is a very valuable step in envisaging the desired fructose fraction in sugar as a function of time. The model

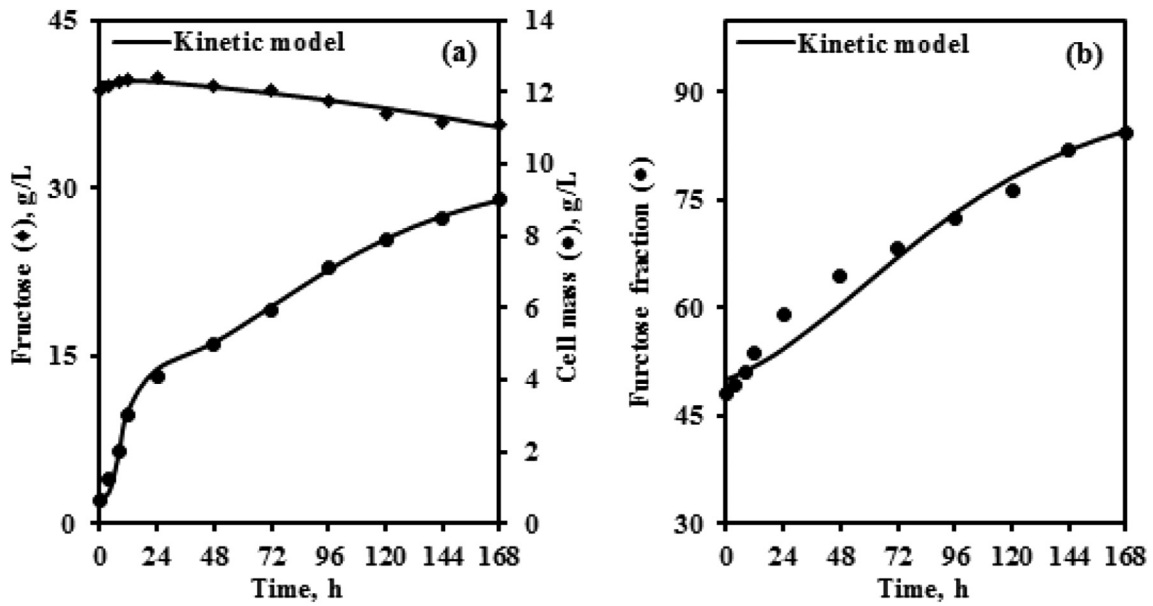


Fig. 3. Kinetic profiles of: (a) cell mass and fructose concentration; (b) fructose fraction; using a new modification to modified Gompertz model.

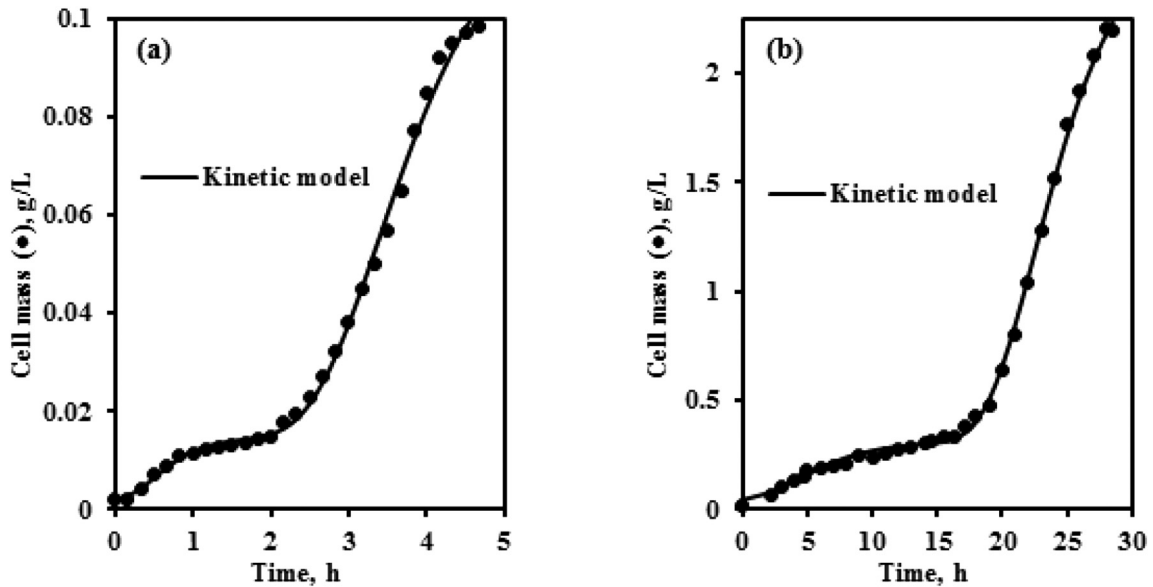


Fig. 4. Kinetic profiles of cell mass of (a) *Streptococcus pneumoniae* on cellobiose and glucose by Boianelli et al. [47]; (b) *Lactococcus lactis* on galactose with addition of nisin by Hartmann et al. [48].

for cell mass predicted excellently the experimental data. The maximum potential for cell mass production ($\gamma_{X,m}$) in the second phase was higher than that in the first phase. However, the rate of

maximum cell mass production ($r_{X,m}$) was higher at first phase; thus affirming the role of inhibition by fructose fraction in the second phase.

Table 4
Predicted parameters of predictive model for cell mass data from literatures.

Products	Parameter	Predicted value		Reference
		First part	Second part	
Cell mass of <i>Streptococcus pneumoniae</i>	$\gamma_{X,m}$ (g/L)	0.0127	0.1108	[47]
	$r_{X,m}$ (g/(L·h))	0.0133	0.0464	
	t_L	0.1630	2.51	
	R^2	0.999		
Cell mass of <i>Lactococcus lactis</i>	$\gamma_{X,m}$ (g/L)	0.2761	2.3843	[48]
	$r_{X,m}$ (g/(L·h))	0.0310	0.2261	
	t_L	1.00	18.67	
	R^2	0.999		

3.6. Validation of proposed model to literature

The proposed model for cell mass concentration prediction was also validated against experimental data from literature [47,48] in which diauxic growth of cell mass occurred. Excellent predictions were obtained for both literatures with the regression coefficients of 0.999 as shown in Fig. 4 and Table 4. The predicted value could confidently be used to describe the real condition as the maximum cell mass ($\gamma_{X,m}$) was 0.0127 in the first stage and 0.1108 in the second stage for reference [47] and 0.2761 in the first stage and 2.3843 in the second stage for reference [48]. The rates of maximum cell mass production ($r_{X,m}$) in the second phase were higher than the rates in the first phase. The short times during high lag phase in the second part were contrary to our work, because diauxic growth on the binary substrates occurred in their works without inhibition from either substrate. The obtained parameters describing the phenomena in the cell growth show that the proposed model is a promising tool to use in other applications of fermentation or biological processes of cell growth.

4. Conclusions

The performance of *S. cerevisiae* in the production of fructose and single cell protein from date syrup showed a potentially promising process. The optimum air flow for cell mass production (10 g/L) was obtained at 1.25 vvm; higher air flow led to lower fructose yield. Lower pH at uncontrolled pH declined the cell growth and lowered the fructose yield; whereas similar final cell mass was obtained in initial pH range of 5–5.6. The yeast growth in controlled pH was inhibited; however, the fructose consumption was suppressed. The proposed model as a modification to the modified Gompertz equation predicted very well the experimental data as well as data from literature. This finding is very useful for further development of process such as high scale or industrial implementation.

Conflicts of interest

The authors declare no conflict of interest.

Financial support

The authors would like to acknowledge the support provided by King Saud University with the Research Supporting Project No. RSP-2019/37 at King Saud University, Riyadh, Saudi Arabia.

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