Optimizing bioethanol production by regulating yeast growth energy

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Abstract The goal of this work is to optimize production of bio-ethanol by fermentation through regulating yeast growth energy (YGE), and provide the mechanism of ethanol production from food-waste leachate (FWL) using yeast (*S. cerevisiae*) as inoculums to be predictable and controllable. The wide range of reduced sugar concentration (RSC) which is commonly administered from low (35 g per liter) to very high (100 g per liter) is responsible for costs increasing besides risks of FWL contamination and death of yeast cells. A mathematical model is presented to describe yeast growth energy (YGE) due to RSC doses along with predicting the amounts of ethanol yield by each dose to identify the optimum one. Simulations of the presented model showed that YGE, energy intake (EI), and their produced ethanol energy (PEE) are always balanced during fermentation process according to the law of conservation of energy. For a better fermentation rate in a continuous process and a large-scale production; YGE should be less than half of EI and more than its quarter (i.e. $\frac{1}{4}EI \leq YGE \leq \frac{1}{2}EI$) which keeps the residual energy less than YGE to avoid risks of osmotic stresses or aging of cells allowing the survival of all yeast cells as long as possible to maximize ethanol production and decrease productivity costs.

Keywords Cell growth energy · Emad formula · *Saccharomyces cerevisiae* · Food waste leachate · Fermentation · Isolated system

Abbreviations

EI	Energy intake
RE	Residual energy
CE	Consumed energy
PPE	Produced ethanol energy
t_D	Doubling time
CGE	Cell growth energy
YGE	Yeast growth energy

Introduction

As yeast ferments different sugars at different rates depending on the process conditions, thus there is a need to optimize the production of alcohol so as to economize the project. Many studies dealing specifically with some of related problems like optimizing ethanol productivity have conducted such topic, but most of them have not introduced a conceptual reasoning to this issue for its statistical analysis nature, or could not show how to predict the ethanol yield for large-scale production. Yet, none of those latter-day scientists could propose a theory or a concept for the mechanism of the fermentation kinematics of such an unlikely appearing event of the amounts of each of reducing sugar concentration (RSC) as an energy intake (EI), and wasted residuals of RSC as an energy loss of the fermentation process. Some parameters are associated with ethanol production in fermentation process such as, temperature, pH, RSC, and ethanol producing micro-organism. Recent studies have investigated the interaction effects of those parameters on the

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production of ethanol by fermentation of food waste leachate (FWL) in the presence of *S. cerevisiae* as inoculums (Man et al. 2011). Temperature exerts a profound effect on all aspects of growth, metabolism, and survival of fermenting organism and fermentation (Xin et al. 2003). Phisalaphong et al. developed a mathematical model to describe the effects of temperature on the kinetic parameters of ethanol fermentation by the flocculating yeast, *Saccharomyces cerevisiae* M30, and using cane molasses as the substrate from the beginning up to the stationary phase (Phisalaphong et al. 2005). Also, effects of pH values of 5.0, 6.0, 7.0 and 8.0 on fermentation were investigated in prior studies concluded that low pH inhibits the yeast multiplication while the inhibitory effect of pH (at the high level) on the ethanol yield could be due to the lower ATP production during the metabolic changes in *S. cerevisiae* (Kadambinigar 2006; Pena et al. 1972). With respect to microorganisms, an understanding of its physiological characteristics including factors that regulate carbon and energy metabolism during growth can furnish useful information when engineering a bioconversion process involving different substrates (Diaz-Ricci et al. 1992; Hill et al. 1993). Various ethanol producing micro-organisms yeast belonging to *S. cerevisiae* have been used most commonly in fermentation where yeast was found to be more ethanol tolerant and produced more ethanol at sugar concentration above 15 % (v/v) (Kadambinigar 2006). Hence, growth yield measurements can provide useful information concerning the relationship among substrate utilization, consumed energy and energy production. Phisalaphong et al. go on to argue that the residual of RSC could be attributed to the occurrence of cell death or inactive conditions including nutritional limitations or high toxic metabolite accumulates without presenting the constraints of such factors (Phisalaphong et al. 2005). One of the main constraints in obtaining higher rates of ethanol production is the inhibition of yeast metabolism by both high concentration of sugar substrate as well as the end product (Douka et al. 1999). Generally in industrial alcohol production an initial of 16–18 % sugar is used and when substrate concentration increases, osmotic pressure becomes pronounced which seriously effects fermentation efficiency (Sprenger 1996).

The relation between cell growth energy (CGE) and cell doubling time (t_D) which is known by Emad formula has been derived and presented by Moawad as follows:

$$CGE = \ln \left[\ln \frac{\ln 2}{t_D} \right]^2 \text{Emad} \quad (1)$$

where

$$\text{Emad} = 23234.59 \text{MeV} \quad (2)$$

in which CGE increases by the increase of cell t_D (Moawad 2010; Moawad 2011a, b; Moawad 2012a, b). Consequently, increasing CGE can be achieved through agents

that lead to the increase of the cell t_D or in other words cause the cell to divide more slowly that if severe or prolonged increases CGE. Current approach aims to investigate the interaction of yeast growth energy (YGE) during the process of fermentation where its increase is favorable and vice versa to avoid osmotic stresses taking into consideration the correlation between growth and aging in yeast; whenever yeast cells grow larger they grow older, *S. cerevisiae* typically divides asymmetrically to give a large mother cell and a smaller daughter cell (Austriaco 1996). As mother cells become old, they enlarge and produce daughter cells that are larger than daughters derived from young mother cells. Like large mothers, large daughter cells have shorter replicative life span (Kennedy 1994). And which confirm that, EI calorie restriction in which nutrient intake is restricted to 60–70 % that of voluntary levels increases life span in most species including mammals (Voet 1586; Tang et al. 2008; Bai et al. 2007). Then, targeting the increase in CGE by increasing the cell t_D should not lead to aging of cells through EI calorie restriction. Consequently, controlling the increase of YGE to avoid the osmotic stress should be through number of yeast cells (n) but not the CGE as $YGE = CGE \times n$. In an effort to assist in the understanding of fermentation and the energy balance that mediate this process, this approach provides a framework for using mathematical techniques to study novel industrial strategies aimed at controlling ethanol production, and tries to relate the RSC course of the treatment to Yeast response during it while maintaining temperature and pH value, and presents the first experimentally driven mathematical model designed to investigate interplay between the possible mechanisms of ethanol production.

Methods and materials

Mathematical model

Evidence is beginning to show that excess of EI may affect fermentation process and in particular, increases ethanol production. The main idea of such hypothesis was concluded from consistent observations have confirmed that energy restriction reduces YGE by inhibiting yeast substrates and vice versa (Yadav et al. 1997). But this interaction between EI and YGE would not last in either direction due to cell death for shorter replicative life span or/and osmotic stress as previously reported. Thus, administering the appropriate EI for ethanol fermentation that minimizes residual energy (RE) and maximizes produced ethanol energy (PEE) can be performed by considering the interaction between the consumed amount of RSC

and the yeast growth in addition to their subsequent of the produced ethanol as an isolated system. Current approach presents a mathematical model of yeast-fermentation process describes YGE during a fermentation process for which efficiency is measured by comparing results of ethanol production to amounts of EI but not for consumed energy (CE). As maintenance of high cell viability is a major characteristic of fermentation to get high ethanol yield, then an important aspect of the model is that YGE should be less than half of EI and more than its quarter ($\frac{1}{4}EI \leq YGE \leq \frac{1}{2}EI$) to avoid risks of either of osmotic stresses or aging of cells. Such double constrain makes RE of the supplied RSC less than that of YGE to keep all yeast cells alive as long as possible to maximize ethanol production and in same time decrease productivity costs. As decreasing YGE to be less than quarter of EI, increases each of costs and risks of cells aging resulting in the productivity decrease. While increasing YGE greater than half of EI, exposes yeast to cells death due to the increasing risks of osmotic stresses as previously explained which minimizes ethanol production. The hypothesis of our model in all cases is that yeast fermentation process is an energy balance process in which summation of YGE and PEE are always in balance with CE, and in same time summation of CE and RE in balance with EI.

Accordingly the above mentioned conditions and constraints can be expressed mathematically as follows:

$$\begin{aligned} \text{Due to balance of energy; } EI &= CE + RE \\ &= [YGE + PEE] + RE, \end{aligned} \quad (3)$$

$$\begin{aligned} \text{Due to cells survival by increasing their life span;} \\ YGE < 1/2EI, \end{aligned} \quad (4)$$

$$\text{Due to osmotic stress; } YGE > RE, \quad (5)$$

$$\begin{aligned} \text{Due to ethanol fermentation efficiency;} \\ [YGE < PEE], [YGE < 1/2(CE)] \end{aligned} \quad (6)$$

Thus from (3) to (6) cases of bio-ethanol production can be expected as follows:

Case I If equations and inequalities from (3) to (6) satisfied then the optimum condition is the one achieves higher productivity in g/L/h.

Case II If $YGE > 1/2[EI]$ and $1/2(RE + 1/2[EI]) < 1/2[CE]$, then yeast cell death will reduce YGE to middle value between RE and $1/2[EI]$ satisfying inequality (6).

Case III If $YGE > 1/2[EI]$ and $1/2(RE + 1/2[EI]) > 1/2[CE]$, then yeast cells would die by higher rates than that of case II to reduce YGE to a value of approximately $1/8(5CE-3RE)$ to satisfy inequality (6).

Case IV If $1/2[EI]$ is twice YGE i.e. $YGE = 1/4[EI]$, organisms saturation point achieved when YGE becomes equivalent to RE, accordingly for higher values of EI over saturation occurs ($RE > YGE$) increasing costs besides to decreasing productivity due to aging of cells as previously explained.

Steps of ethanol production by yeast fermentation: FWL was collected from food waste resource recovery plant, *Saccharomyces cerevisiae* was used in all experiments as illustrated by Le Man et al. (2008). The strain was cultivated in the growth medium for a period of 48 h, where cell concentration of the yeast was checked by both its optical density at 600 nm value on a BioRite spectrophotometer and a counting chamber (Fisher Scientific, Fairlawn, NJ). It was kept under sterile conditions, at neutral pH and 35 °C in an incubating shaker run at 150 rpm.

Cell concentration in the cultivated medium was 2.3×10^8 Cells/mL. The growth medium used for cultivation of inoculum culture consisted of glucose 10 g/L, yeast extract 3 g/L, malt extract 3 g/L and peptone 3 g/L as conducted by Nahvi et al. (Nahvi et al. 2002). Thereafter, Collected FWL was thoroughly blended in a mixer and then sterilized in an autoclave at 120 °C for 15 min. The pH was adjusted using 1N H₂SO₄ and 1N NaOH and RSC was adjusted either by diluting with water or by adding glucose. Batch experiments were carried out in a series of 500 mL Erlenmeyer flasks, containing 200 mL of FWL, 2.5 % inoculum culture was dispensed to each flask. Added concentrations of RSC to these batch experiments were varied between 35 and 100 g/L, while the temperature and pH were maintained at 38 and 5 °C respectively according to prior studies for optimizing bio-ethanol production by yeast fermentation (Man et al. 2011; Phisalaphong et al. 2005; Kadambiniguar 2006). The flasks were shaken at 180 rpm in a thermostat controlled incubating shaker under the designed temperatures for 40 h. To evaluate the potential of ethanol yield from FWL, experiments were conducted according to the parameters of the presented mathematical model that predominantly affect the ethanol yield in a fermentation process which are EI, CE, RE and YGE. To study the combined effects of EI factor, experiments were performed according to prior statistically designed experiments using 35, 45, 60, 75, 85, and 100 g/L of RSC ($p \leq 0.001$) (Le Man et al. 2010). The total solids and volatile solids concentrations of samples were estimated according to the procedure outlined in standard methods (APHA 1998). RSC was determined using the standard dinitrosalicylic acid method (Miller 1959). While, estimation of ethanol concentration can be done as follows: Ten mL of fermentation broth was centrifuged at 5,000 rpm for 30 min at 10 °C and the supernatant was used to estimate the ethanol concentration. Ethanol was

analyzed by gas chromatography (HP-5960, Global Medical Instrumentation Inc, MN, USA) equipped with CP-Wax column at 110 °C, flame ion detector (FID) 220 °C and injector 170 °C. Nitrogen was used as carrier gas at a flow rate of 30 cm³/min and the combustion gas was a mixture of hydrogen and air. For data analysis, ethanol concentration at the end of a 25 h test period correspond to the presented ascending values of RSC were 7.64, 10.7, 12.93, 18.7, 22.63, and 26.81 g/L respectively ($p \leq 0.001$). While the corresponding residual amounts of RSC were 10.13, 12.5, 17.353, 20, 25.4, and 37.5 g/L, after continuous periods of 9, 11, 12.5, 17, 21, and 25 h respectively ($p \leq 0.001$). Since, experiment done by maintaining temperature and pH value, the fermentation process had been affected by other variable parameters i.e. the balance between CE and YGE. Knowing that number of cells (C_0) was constant for all batches in the presence of 2.5 % of *S. cerevisiae* as inoculums:

Thus, $C_0 = \frac{2.5}{100} \times 200\text{mL} \times 2.3 \times 10^8 \frac{\text{Cells}}{\text{mL}} = 5 \times 2.3 \times 10^8$ Cells. Growth of *S. cerevisiae* was measured by optical density method using a spectrophotometer (Kadambinugar 2006), where estimating YGE for each RSC were computed in Emad unit by using Eq. (1) considering yeast growth constant ($\ln 2/tD$) equivalent to RSC reducing constant ($\ln 2/t_{1/2}$). Then using Eq. (2), value of YGE can be converted into MeV, and then into Joules to express energies of all considered factors of fermentation process in current approach in same unit (Joule), knowing that energy per one gram of each of RSC and ethanol are 17 and 29 J/g respectively.

Result and analysis

To estimate the maximum ethanol production potential of fermentation process for certain amount of EI supplied to 1 L of FWL inoculated by certain amount of *S. cerevisiae*, the previous data of observations and results of bioethanol production for different RSC concentrations in the presence of constant percentage (2.5 %) of *S. cerevisiae* as FWL inoculum is tabulated in Table 1 and illustrated in Fig. 1 (Moawad 2011b).

Predicted ethanol concentration for 35 g/L of RSC

Per 200 mL; EI = 7×17 J; After 9 h: RE = 2×17 J;
CE = 5×17 J

Half-life time of decreasing

$$\text{RSC } (t_{1/2}) = \frac{9}{\log_2[7/2]} = 4.98\text{h.}$$

From Eq. (1)

Table 1 Observations and results of bioethanol production for different RSC concentrations in the presence of constant percentage (2.5 %) of *S. Cerevisiae* as FWL inoculum (Moawad 2011b)

RSC (g/L)	Residual amounts of RSC (g/L)	Produced ethanol (g/L)	Period of process (H)	Productivity in g/L/h
035	10.130	07.69	09.0	0.854
045	12.500	10.99	11.0	0.999
060	17.353	12.99	12.5	1.040
075	20.000	19.00	17.0	1.118
085	25.400	23.12	21.0	1.101
100	37.500	27.00	25.0	1.080

Fig. 1 Shows productivity of Bioethanol in g/L/h via concentrations of RSC in g/L (Moawad 2011b)

$$\text{CGE} = \ln \left[\ln \left(\frac{\ln 2}{4.98 \times 60 \times 60} \right) \right]^2 = 4.637 \text{ Emad.}$$

From Eq. (2)

$$\text{YGE} = 5 \times e^{\frac{\ln 2}{4.98} \times 9} \times 2.3 \times 10^8 \times 4.637 \times \frac{23234.59}{6.242 \times 10^{12}} = 69.47\text{J} > 1/2[\text{EI}],$$

which contradicts the hypothesis of the presented model through inequalities (5) and (6) as in Case III. Consequently, yeast cells will be exposed to high death rate led to reduce YGE until satisfaction of model inequalities (4), (5) and (6) i.e. $\text{RE} < \text{YGE} < 1/2[\text{EI}]$ provided that $\text{YGE} < 1/2[\text{CE}]$ and get balanced according to Case III at $\text{YGE} = 1/8(5\text{CE}-3\text{RE}) = 40.375$ J. Thus from Eq. (3), ethanol concentration for RSC of 35 g/L can be predicted according to case III as follows; $\text{PEE} = \text{CE}-\text{YGE} = 44.625$ J/200 mL = 223.125 J/L = 7.69 g/L, which is 98 % identical to the average value of the observed ethanol concentration for RSC of 35 g/L.

Predicted ethanol concentration for 45 g/L of RSC

Per 200 mL; EI = 9×17 J; After 11 h: RE = 2×17 J;
CE = 7×17 J.

Half-life time of decreasing

$$\text{RSC}(t_{1/2}) = \frac{11}{\log_2[9/2]} = 5.07 \text{ h.}$$

From Eq. (1)

$$\text{CGE} = \ln \left[\ln \left(\frac{\ln 2}{5.07 \times 60 \times 60} \right) \right]^2 = 4.64 \text{ Emad.}$$

From Eq. (2)

$$\begin{aligned} \text{YGE} &= 5 \times e^{\frac{\ln 2}{5.07} \times 11} \times 2.3 \times 10^8 \times 4.64 \times \frac{23234.59}{6.242 \times 10^{12}} \\ &= 89.38 \text{ J} > 1/2[\text{EI}], \end{aligned}$$

which contradicts the hypothesis of the presented model through inequality (5).

Consequently, yeast cells will be exposed to death that led to reduce YGE until satisfaction of model inequalities (5) and (4) i.e. $\text{RE} < \text{YGE} < 1/2[\text{EI}]$.

Since, $1/2(\text{RE} + 1/2[\text{EI}]) < 1/2[\text{CE}]$, then according to Case II, YGF would be balanced at middle value of such range i.e.

$$\text{YGE} = \frac{[(2 \times 17) + \frac{1}{2}(9 \times 17)]}{2} = 55.25 \text{ J.}$$

Accordingly, ethanol concentration for RSC of 45 g/L can be predicted according to case II as follows; $\text{PEE} = \text{CE} - \text{YGE} = 63.75 \text{ J}/200 \text{ mL} = 318.75 \text{ J/L} = 10.99 \text{ g/L}$, which is 98 % identical to the average value of the observed ethanol concentration for RSC of 45 g/L.

Predicted ethanol concentration for 60 g/L of RSC

Per 200 mL; EI = $12 \times 17 \text{ J}$; After 12.5 h: RE = $3.47 \times 17 \text{ J}$; CE = $8.53 \times 17 \text{ J}$.

Half-life time of decreasing

$$\text{RSC}(t_{1/2}) = \frac{12.5}{\log_2[12/3.47]} = 6.98 \text{ h.}$$

From Eq. (1)

$$\text{CGE} = \ln \left[\ln \left(\frac{\ln 2}{6.98 \times 60 \times 60} \right) \right]^2 = 4.703 \text{ Emad.}$$

From Eq. (2)

$$\begin{aligned} \text{YGE} &= 5 \times e^{\frac{\ln 2}{6.98} \times 12.5} \times 2.3 \times 10^8 \times 4.703 \times \frac{23234.59}{6.242 \times 10^{12}} \\ &= 69.66 \text{ J} < 1/2[\text{EI}], \end{aligned}$$

which satisfies the hypothesis of the presented model through inequality (5).

Consequently, ethanol concentration for RSC of 60 g/L can be predicted according to case I as follows; $\text{PEE} = \text{CE} - \text{YGE} = 75.35 \text{ J}/200 \text{ mL} = 376.75 \text{ J/L} = 12.99 \text{ g/L}$, which

is 99.5 % identical to the average value of the observed ethanol concentration for RSC of 60 g/L.

Predicted ethanol concentration for 75 g/L of RSC

Per 200 mL; EI = $15 \times 17 \text{ J}$; After 17 h: RE = $4 \times 17 \text{ J}$; CE = $11 \times 17 \text{ J}$.

Half-life time of decreasing

$$\text{RSC}(t_{1/2}) = \frac{17}{\log_2[15/4]} = 8.9 \text{ h.}$$

From Eq. (1)

$$\text{CGE} = \ln \left[\ln \left(\frac{\ln 2}{8.9 \times 60 \times 60} \right) \right]^2 = 4.7485 \text{ Emad.}$$

From Eq. (2)

$$\begin{aligned} \text{YGE} &= 5 \times e^{\frac{\ln 2}{8.9} \times 17} \times 2.3 \times 10^8 \times 4.7485 \times \frac{23234.59}{6.242 \times 10^{12}} \\ &= 76.4 \text{ J} < 1/2[\text{EI}], \end{aligned}$$

which satisfies the hypothesis of the presented model through inequality (5).

Consequently, ethanol concentration for RSC of 75 g/L can be predicted according to case I as follows; $\text{PEE} = \text{CE} - \text{YGE} = 110.6 \text{ J}/200 \text{ mL} = 553 \text{ J/L} = 19 \text{ g/L}$, which is 98 % identical to the average value of the observed ethanol concentration for RSC of 75 g/L.

Predicted ethanol concentration for 85 g/L of RSC

Per 200 mL; EI = $17 \times 17 \text{ J}$; After 21 h: RE = $5 \times 17 \text{ J}$; CE = $12 \times 17 \text{ J}$.

Half-life time of decreasing

$$\text{RSC}(t_{1/2}) = \frac{21}{\log_2[17/5]} = 11.89 \text{ h.}$$

From Eq. (1)

$$\text{CGE} = \ln \left[\ln \left(\frac{\ln 2}{11.89 \times 60 \times 60} \right) \right]^2 = 4.8 \text{ Emad.}$$

From Eq. (2)

$$\begin{aligned} \text{YGE} &= 5 \times e^{\frac{\ln 2}{11.89} \times 21} \times 2.3 \times 10^8 \times 4.8 \times \frac{23234.59}{6.242 \times 10^{12}} \\ &= 70 \text{ J} < 1/2[\text{EI}], \end{aligned}$$

which satisfies the hypothesis of the presented model through inequalities (5) and (6).

Consequently, ethanol concentration for RSC of 85 g/L can be predicted according to case I as follows; $\text{PEE} = \text{CE} - \text{YGE} = 134 \text{ J}/200 \text{ mL} = 670 \text{ J/L} = 23.12 \text{ g/L}$, which is 98 % identical to the average value of the observed ethanol concentration for RSC of 85 g/L.

Predicted ethanol concentration for 100 g/L of RSC

Per 200 mL; EI = 20×17 J; After 25 h: RE = 7.5×17 J; CE = 12.5×17 J.

Half-life time of decreasing

$$\text{RSC } (t_{1/2}) = \frac{25}{\log_2[20/7.5]} = 17.67\text{h.}$$

From Eq. (1)

$$\text{CGE} = \ln \left[\ln \left(\frac{\ln 2}{17.67 \times 60 \times 60} \right) \right]^2 = 4.872 \text{ Emad.}$$

From Eq. (2)

$$\text{YGE} = 5 \times e^{\frac{\ln 2}{17.67} \times 25} \times 2.3 \times 10^8 \times 4.872 \times \frac{23234.59}{6.242 \times 10^{12}} \\ = 55.61 \text{ J} < 1/4[\text{EI}].$$

Consequently, ethanol concentration for RSC of 100 g/L can be predicted according to case IV as follows; PEE = CE-YGE = $156.9 \text{ J}/200 \text{ mL} = 784.47 \text{ J/L} = 27 \text{ g/L}$, which is 99 % identical to the average value of the observed ethanol concentration for RSC of 100 g/L.

Optimum condition

Table and Fig. 2 show the results of the presented physical analysis of bioethanol production for different RSC concentrations in the presence of constant percentage of *S. cerevisiae* (2.5 %) as FWL inoculum.

RSC concentrations of (60, 75, 85 g/L) of case I, satisfied hypothesis of the presented mathematical model through equations and inequalities from (3) to (6) then the optimum condition was the one of those RSC concentrations that yielded higher productivity in g/L/h. Thus, the optimum condition was achieved by 75 g/L of RSC where maximum ethanol concentration obtained in the presence of 2.5 % of *S. cerevisiae* as inoculums was 1.118 g/L/h.

Fig. 2 Shows balance of energies during bioethanol production by fermentation for different RSC concentrations in the presence of constant percentage (2.5 %) of *S. Cerevisiae* as FWL inoculum

Estimating model for ethanol production

The observed experimental results were evaluated using analysis of variance (ANOVA) in order to identify the influence of RSC levels on the ethanol production in the presence of maintaining each of pH value, temperature, and cell concentration of the yeast at 5, 38 °C and 2.3×10^8 Cells/mL respectively. ANOVA showed a considerable significance of RSC level ($p < 0.001$) and high positive influence on the ethanol production (EP) ($p < 0.001$), explained by the following efficient estimating model: $\text{EP} = 0.11 \times (\text{RSC})^{1.19312661} \text{ g/L}$ (7) with good fit ($R^2 = 0.98$) could be easily applied for scale—up to produce value—added ethanol and to confirm and provide a clear-cut criterion for accepting the hypothesis of regarding bioethanol production by fermentation as an energy balance process.

Discussion

This research lays down the basis for optimizing bio-ethanol production through regulating YGE, where the controversial objectives of the current approach are (1) estimating the maximum ethanol production potential of inoculated FWL, (2) minimizing energy loss and (3) understanding the main and interactive effects of YGE parameter on fermentation process and its role in the administered RSC that could lead to minimize its residual and in same time to maximize its ethanol production. It is evident that increasing RSC (EI) leads to YGE increasing and consequently ethanol production is optimized until fermentation process stopped as soon as RSC energy becomes smaller than that of yeast growth energy for substrate inhibition due to osmotic stress which arises from the sudden change in the solute concentration around a cell, causing a rapid change in the movement of water across its cell membrane. Under conditions of high concentrations of either salts, substrates or any solute in the supernatant, water is drawn out of the cells through osmosis. This also inhibits the transport of substrates and cofactors into the cell thus “shocking” the cell. Alternatively, at low concentrations of solutes, water enters the cell in large amounts causing it to swell and either burst or undergoes apoptosis (Lang et al. 2005). Since EI and YGE vary inversely to each other during fermentation process then ratio between them at the beginning should be managed such that YGE to be lower than EI but higher than RE by the end of the fermentation process to avoid the sudden change in the solute concentration around cells along the whole process.

Maximum ethanol concentration of 26.81 g/L was obtained at the condition of temperature (38 °C), pH (5) and RSC (100 g/L), while the ethanol concentration at the

optimum experimental condition 8.77 g/L obtained at the condition of temperature (38 °C), pH (5) and RSC (75 g/L) results in highest productivity (1.118 g/L/h), and agreed well with the predicted one (19 g/L). Such accuracy indicates the suitability of the model employed to optimize the conditions of ethanol production from FWL. Corresponding to the ethanol concentration of 18.77 g/L, the ethanol yield from the FWL was obtained as 0.25 g ethanol/g reducing sugar which appears efficient by 25 %, but in fact with respect to produced energy efficiency is 42.6 % as energy of 1 g ethanol is 29 J, while that of sugar is 17 J only. In the present study, *S. cerevisiae* was chosen as the ethanol—producing microbial strain. The selection of this strain was according to the investigation carried out by previous researchers using *S. cerevisiae* for the fermentation of malt spiked with trichothecenes, which was a highly contaminated substrate (Garda et al. 2005). The growth of *S. cerevisiae* in gradually increasing concentrations of sugar showed an increase in optical density up to 75 g/L sugar concentration in medium as shown in Table 2. However, on increasing the sugar concentration beyond 75 g/L, the growth was inhibited as shown by the measured optical density. The potential of open and closed fermentation processes for ethanol production from FWL has been shown with an optimum ethanol concentration of 19 g/L, under optimal conditions of time (17 h), pH (5) and temperature (38 °C). Developing the presented mathematical model has showed the importance of estimating the limits of EI that required for either of growing life organisms and increasing their life span, or inhibiting their growth rate that lead to shorter replicative life span due to cell aging. In either case the growth energy of such life organisms should be calculated. This revealed the importance of Emad formula, which calculates the growth energy of the growing systems that follows the exponential growth by knowing its growth constant (Moawad 2010; Moawad 2011a, b; Moawad 2012a, b). This allowed for the first time to measure the biological growth energy in MeV or Joules, to assess the limits of energy that suitable for the fermentation process. Most of current studies focus mainly on statistical models to estimate the optimum RSC for a fermentation process. Although such models can give good results, they lack the predictive accuracy of conceptual reasoning, in addition to the numerous costs and long time of such statistical trails. Let us imagine how researchers reach the proper sugar concentration through the statistical analysis to determine the best operating condition, to yield high ethanol production, at both low and high setting of the process parameters, despite that they could reach same results directly through the introduced physical analysis. For instance, Le Man et al. (2008) identified the useful quantities utilized in their interesting application by statistical model, in which statistical interpretation of the results done in terms of Fischer's variance ratio (F) and probability values (p), and finally haven't considered that further studies leading to determine level of EI that would induce cell killing are

Table 2 Physical analysis of Bioethanol production for different RSC concentrations in the presence of constant percentage (2.5 %) of *S. Cerevisiae* as FWL inoculum (Moawad 2011b)

Case	RSC (g/L)	YGE (J)	Produced ethanol (g/L)	Productivity in g/L/h
YGE > 0.5 EI & [RE + 0.5 EI] > CE III	035	40.375	07.69	0.854
YGE > 0.5 EI & [RE + 0.5 EI] < CE II	045	55.250	10.99	0.999
YGE < 0.50 EI I	060	70.100	12.99	1.040
YGE < 0.50 EI I	075	76.400	19.00	1.118
YGE < 0.50 EI I	085	70.300	23.12	1.101
YGE ≤ 0.25 EI IV	100	52.300	27.00	1.080

strongly warranted. This encouraged the author of this paper to focus on that missing point in the presented model of current physical approach, which is considered a practical proof for the assessment of law of conservation-energy for yeast fermentation as an isolated system. Accuracy of current physical method has been checked and compared with the statistical method used in several experimental treatments conducted in different biotechnology societies. Results of both methods were 97–100 % identical. Such promising identically have urged the author of this study to depend on the statistical analysis of those great scientists (Man et al. 2011; Xin et al. 2003; Phisalaphong et al. 2005; Kadambinigar 2006; Pena et al. 1972; Diaz-Ricci et al. 1992; Hill et al. 1993; Douka 1999; Sprenger 1996; Moawad 2010; Moawad 2011a, b; Moawad 2012a, b; Austriaco 1996; Kennedy 1994; Voet 1586; Tang et al. 2008; Bai et al. 2007; Yadav et al. 1997; Le Man et al. 2008; Nahvi et al. 2002; Le Man et al. 2010; APHA 1998; Miller 1959; Lang et al. 2005; Garda et al. 2005) on purpose, regarding their results as a “gold standard” or fully accepted measurement against which to make a comparison to determine accuracy of the approach.

Conclusion

Better fermentation rate and efficiency in a continuous process can be performed by administering an appropriate EI for ethanol fermentation able to minimize RE and to maximize PEE through considering the interaction between EI, YGE, and their subsequent PEE as an energy balance process of isolated system. As maintenance of high cell

viability is a major characteristic of fermentation to get high ethanol yield, thus YGE should be less than half of EI and more than its quarter ($\frac{1}{4}EI \leq YGE \leq \frac{1}{2}EI$) which keeps RE less than YGE to avoid risks of osmotic stresses or aging of cells allowing the survival of all yeast cells as long as possible to maximize ethanol production and decrease productivity costs.

Conflict of interest The author declares that there is no conflict of interest concerning this paper.

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