Accepted Manuscript

Title: Aeration-enhanced bioethanol production

Author: Irem Deniz Esra Imamoglu Fazilet Vardar-Sukan

PII: S1369-703X(14)00126-0
DOI: http://dx.doi.org/doi:10.1016/j.bej.2014.05.011
Reference: BEJ 5946

To appear in: *Biochemical Engineering Journal*

Received date: 3-3-2014
Revised date: 2-5-2014
Accepted date: 7-5-2014

Please cite this article as: I. Deniz, E. Imamoglu, F. Vardar-Sukan, Aeration-enhanced bioethanol production, *Biochemical Engineering Journal* (2014), http://dx.doi.org/10.1016/j.bej.2014.05.011

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Aeration-Enhanced Bioethanol Production

Irem DENIZ[1, 2*]; Esra IMAMOGLU[1]; Fazilet VARDAR-SUKAN[1]

[1]*Department of Bioengineering, Faculty of Engineering, Ege University, 35100 Bornova-Izmir, Turkey*

[2]*Department of Bioengineering, Faculty of Engineering, Celal Bayar University, Muradiye-Manisa, Turkey*

[*]Corresponding author

Tel: +90 232 388 49 55

Fax: +90 232 388 49 55

E-mail address: iremdenz@gmail.com (Irem DENIZ)

Accepted Manuscript

ACCEPTED MANUSCRIPT

Abstract

In recent years, growing attention has been devoted to maximize the product yield for the conversion of biomass into ethanol. In this study, microaerated conditions were established to enhance the ethanol yield by *Eschericia coli* KO11. According to the results, limited aeration was found to be an important factor to increase the ethanol yield by improving the consumption of sugars and the production of biomass. The best result was obtained using oxygen transfer rate (OTR) of 5 mmol/L/h, reaching 19.66 g/L of ethanol at 48 h using quince pomace as substrate. The assays showed that less than 5% of the initial sugar remained at the end of the fermentation, achieving a biomass concentration of 7.3 g/L. In conclusion, we successfully carried out lab-scale production of bioethanol from quince pomace using the ethanologenic *E. coli* KO11. In particular, microaerobic ethanol fermentation at OTR= 5 mmol/L/h is suggested for the efficient utilization of sugars in quince pomace. Considering the abundance of raw material and the ease of large-scale production, this improvement will have a considerable impact on the total cost of bioethanol.

Keywords: Aeration; ethanol; *E. coli* KO11; bioreactors; oxygen transfer; batch processing.

Accepted Manuscript

ACCEPTED MANUSCRIPT

1. Introduction

Ethanol is considered as one of the best alternative energy resources because it reduces the CO_2 emission in to the air [1]. In this regard, bioconversion of biomass to ethanol has been receiving a great amount of interest worldwide. In order to promote bioethanol utilization, it is necessary to reduce the production cost using agroindustrial wastes. Conventionally, bioethanol has been produced by physiochemical treatment of the biomass containing multi glucose units, using microbial catalysis and metabolic engineering under unaerated conditions [2]. As a promising biocatalyst for ethanol production from agroindustrial wastes, various ethanologenic recombinant *Escherichia coli* strains have been studied intensively by many researchers. In particular, *E. coli* KO11 has been employed as a promising biocatalyst to convert hemicellulosic waste into ethanol, because *E. coli* is capable of assimilating fructose and sucrose as well as glucose, which are the predominant sugars accounting for of 99% of dried quince pomace [3]. The strain has a higher product yield of 0.4 g/L under unaerated conditions [4]. However it was shown that under microaerated conditions the ethanol yield reached to 0.47 g/L with a 25% higher sugar uptake ratio in comparison to unaerated conditions [5].

The gas–liquid mass transfer and oxygen uptake rate are strongly influenced by the hydrodynamic conditions in the bioreactors. These conditions are known to be a function of energy dissipation that depends on the operational conditions, the physicochemical properties of the culture, the geometrical parameters of the bioreactor and also on the presence of oxygen consuming cells. Oxygen mass transfer controls the performance of bioreactors. Successful design depends on the comprehension and elucidiation of the complex hydrodynamic interactions within the reactor [6].

Aeration conditions influence mass transfer by affecting the bubble size, air hold up and turbulance within the vessel as well as biomass production. A wide range of environmental conditions are required to obtain maximum productivity in a bioreactor depending on the specific process. Attempts of optimization with respect to productivity and process economics should take into account design variables such as aeration capacity, sparger type and etc.

From the viewpoint of the industrial-scale production of bioethanol, it is a very simple and practical operation to control fermentation performance through the oxygen transfer rate (OTR), because the OTR can theoretically be controlled by adjusting both aeration rates and

Accepted Manuscript

ACCEPTED MANUSCRIPT

durations. In this study, the effect of aeration and OTR on the fermentation performance was investigated as an operational parameter for the control of pilot scale bioethanol production. Cemeroglu and Karadeniz [3] reported that the quince pomace was directly used as a substrate without any chemical pretreatment (such as acid or base hydrolysis) due to the availability of the sugars (mostly glucose and fructose) in the pomace for microorganisms. There are several studies on the utilization of the fruit pomaces such as apple pomace without pretreatment process for the bioconversion of value-added bioproducts [7-9]. In this study quince pomace as an agro-industrial biomass was used for bioethanol production under microaerated conditions, eliminating the pretreatment step. To our knowledge, this is the first report on the successful utilization of quince pomace for bioethanol production on the lab scale fermentation under microaerated conditions. Also, the existing micoaeration studies using *E. coli* KO11 are conducted using full aeration during fermentation which requires more air feeding and power consumption.

2. Materials and Methods

2.1. Growth Conditions

Recombinant *E.coli* KO11 (pLOI 1910) strain was provided by courtesy of Professor L.O. Ingram from University of Florida. Stock cultures were stored in 20% glycerol at –86 °C. Working cultures of KO11 were maintained on modified Luria-Bertani (LB) agar containing (per liter) 5 g of NaCl, 5 g of yeast extract, 10 g of tryptone, 20 g of glucose, 15 g of agar, and 600 mg of chloramphenicol at 4 °C.

For inoculation, cells from 3 fresh colonies were transferred into 500 ml flasks containing 150 ml LB medium supplemented with 50 g/L glucose. Seed cultures were incubated under static conditions for 16 h at 30 °C. Cells were harvested by centrifugation (5000 g, 5 min, 5°C) and washed with the fermentation medium. The initial cell density was adjusted to the given concentration range of 0.33 g-dry cell weight/L. No chloramphenicol was included in seed cultures or fermentations.

2.2. Substrate

Quince pomace was used as a substrate for ethanol production instead of glucose. Quinces were pressed and dried to constant weight at 70°C in a pasteur oven (Memmert, Germany) to

Accepted Manuscript

ACCEPTED MANUSCRIPT

remove bound-water. Dried pomace was grinded to 0.1 mm in size. No further pretreatments were carried out.

The total carbon (C) and nitrogen (N) contents of dried quince pomace were determined by the methods described elsewhere [10, 11] and are shown in Table 1. Quince pomace was added to the reactor as carbon source to give a C:N ratio of 14.33 g/g, which was determined based on the elemental composition of the LB medium supplemented with glucose.

2.3. Reactor Conditions

Batch fermentations were carried out in 5 L (Sartorius A plus stat.) bioreactors with a working volume of 2 L, containing quince pomace and LB broth without glucose. Quince pomace and the supported LB ingredients were autoclaved separately and mixed aseptically before fermentation. The fermentation was carried out at constant pH 5.5 at 35 °C. 2 M KOH solution was automatically added to prevent acidification. No antifoam or antibiotic were used in reactor experiments. All experiments were carried out in duplicate.

2.3.1. Aeration and k_La determination

Different aeration rates and different aeration durations were studied with quince pomace supported LB medium in order to determine the influence of oxygen addition on sugar consumption and ethanol production by *E. coli* KO11. Thus, a one factor at a time design was used. For the first set of experiments, different aeration rates of 0, 0.02, 0.035, 0.047 and 0.062 vvm were fed to the reactor for the first 8 hours of the fermentation, corresponding to OTR values of 0, 2, 5, 9, 16 mmol/L/h, respectively. The results were compared in terms of product yield for the fermentation period of 64 h. For the second set, 0.035 vvm air was fed to the reactor for the first 6, 8, 10 and 12 hours to determine the most appropriate aeration duration for the ethanol production period of 64 h.

The volumetric oxygen transfer coefficient, k_La, was determined using a polarographic oxygen sensor calibrated with nitrogen and air sparging to set zero and 100%, respectively. The determination of the k_La values was done following the unsteady state method of gassing out using Eq. 1 [12];

$$OTR = dO_2/dt = k_La(C^* - C_L) - q_{O_2}X \quad (1)$$

Accepted Manuscript

ACCEPTED MANUSCRIPT

where k_La is the oxygen mass transfer coefficient; C^* is the dissolved oxygen concentration at saturation in the bulk; C_L is the dissolved oxygen concentration at any time; q_{O2} is the specific oxygen uptake rate and X is the biomass concentration.

2.3.2. Mathematical equations

Mixing time was experimentally determined using the pH-response technique [13]. Mathematical equations in order to determine rheological behavior of the reactor for bioethanol production are presented in Table 2. For stirred-tank bioreactors, the power numbers were obtained from Power Number (N_P) curve as a function of the impeller Reynolds number (Re) and the impeller type [14]. The characteristic empirical constant (k) for a standard Rushton turbine impeller was taken as 10 [15]. The rheological behavior of fermentation broth is summarized in Table 3.

2.4. Analytical Measurements

Biomass was determined and validated by counting colony forming units, measuring absorbance and dry cell mass. Absorbance was measured at 600 nm (A_{600}) using a Unicam-Helios-α spectrophotometer, and cell concentration was calculated and converted to g-dry cell mass per liter (DC*m*/L) using the conversion coefficient of 0.33 g-DC*m*/L/A_{600}, for *E. coli* KO11.

Assuming that cell death was negligible, the biomass formation rate (μ) was calculated (Eq. 2) and maximum specific growth rate (μ_{max}) was determined with regards to maximum cell concentration derivation of two samples [16].

$$\mu = \frac{\ln X_2 - \ln X_1}{\Delta t} \tag{2}$$

where X_2 is the final cell concentration, X_1 is the initial cell concentration and Δt is the time required for the increase in concentration from X_1 to X_2.

The fermentation broth viscosity was measured twice, at the beginning and at the end of the fermentation period by a rotational viscometer (Brookfield model DV-E, USA) with a LV type spring torque using a LV1-61 spindle and was determined by Poiseuille equation (Eq. 3).

$$\frac{dV_L}{dt} = \frac{\pi D_C}{8\eta}\frac{dP}{L} \tag{3}$$

Accepted Manuscript

ACCEPTED MANUSCRIPT

where dV_L/dt is the volumetric flow rate of the liquid, D_C is the radius of the pipe, dP is the pressure drop, L is the length of pipe and η is the dynamic viscosity. An average viscosity of 1.36×10^{-6} m^2/s was used in the equations. The density of the fermentation broth was measured by 25 mL pycnometer (Isolab, Germany) at the beginning and at the end of the fermentation and an average density value was used in the study (1033 kg/m^3).

The total reducing sugar content of quince pomace was determined using the dinitrosalicylic acid (DNS) method, where the absorbance was measured at 540 nm [17]. The sugar uptake ratio was defined as the percentage of the total amount of sugar consumed during fermentation over the total sugar at the start of fermentation. The specific sugar consumption was calculated as the amount of sugar consumed at any time over the produced biomass concentration.

Ethanol concentrations were measured using a Gas Chromatograph (6890N Agilent Technologies Network GC System) equipped with a flame ionization detector and a DB-FFAP 30 m × 0.32 mm × 0.25 mm capillary column (J&W Scientific) [18].

The ethanol yield ($Y_{P/S}$) was defined as the amount of ethanol produced per the amount of sugar consumed during fermentation (Eq. 4). Total ethanol yield against theoretical yield, specific ethanol yield ($Y_{P/X}$) and volumetric productivity (Q_P) were calculated by the Eq. 5, 6 and 7, respectively.

#

$$Y_{P/S} = \frac{dE}{dS} \tag{4}$$

$$Y_{P/X} = \frac{dE}{dX} \tag{5}$$

$$Total\ ethanol\ yield = \frac{dE}{dS} x \frac{1}{0.51} x100 \tag{6}$$

Accepted Manuscript

ACCEPTED MANUSCRIPT

$$Q_p = \frac{dE}{dt} \quad (7)$$

where *dE*, *dS and dX* are the total ethanol production, sugar consumption and biomass production during the fermentation, respectively.

2.5. Statistical Analyses

Statistical analyses of the data were performed by one-way analysis of variance (ANOVA). A probability value of $p<0.05$ was considered to denote a statistically significant difference of two batches. Data are presented as mean values ± SEM (standard error of the mean).

Accepted Manuscript

ACCEPTED MANUSCRIPT

3. Results and Discussion

In order to explore the operating parameters affecting the bioethanol production from quince pomace, microaerated conditions were established at different aeration rates for variable aeration durations. Hydrodynamic parameters and kinetic parameters, such as ethanol yield, sugar uptake ratio and specific growth rate, were compared to those obtained in unaerated conditions.

3.1. Effect of aeration rate

In order to understand the process of the bioethanol production by *E. coli* KO11, firstly, an anaerobic fermentation was conducted and then compared with microaerated fermentation in a 5 L stirred tank bioreactor. Fig 1 shows the time courses of the fermentation conducted at OTR values of 0 (unaerated), 2, 5, 9 and 16 mmol/L/h. Ethanol production reached 17.15 g/L in 48 hours under unaerated conditions. As a result of the limiting oxygen concentration, the dissolved oxygen concentration dropped below 5% after 8 h of the fermentation, which resulted in 24.81% remaining sugar with respect to the initial sugars under unaerated conditions. As illustrated in Table 4, *E. coli* KO11 did not consume the reduced sugars in the quince pomace effectively during the anaerobic fermentation. The inefficient use of sugars resulted in lower ethanol yields. This was shown by the sugar uptake ratios which were promoted under aerated conditions.

The final ethanol concentration increased to a maximum of 20.51 g/L at the OTR value of 5 mmol/L/h in 64 h. Moreover, the cells produced ethanol with an overall yield of 0.33 g ethanol/g sugar. The maximum sugar uptake ratio was also obtained at OTR= 5 mmol/L/h (Table 4). Microaeration enhanced the ethanol yield and the productivity via promoting sugar utilization in the laboratory-scale fermentation of quince pomace compared to the unaerated culture. As reported by Okuda et al. [5], the microaerobic condition increased NAD supply, which enhanced the glucolytic flux including the pathway toward phosphoenolpyruvate, resulting in an increase in the rate of glucose transport. The results of the study agree with

Accepted Manuscript

ACCEPTED MANUSCRIPT

Agbogbo et al. [19], Bellido et al. [20] and Lin et al. [21], who concluded that the higher sugar uptake ratio leaded to higher ethanol production.

Table 4 also shows the kinetic parameters for each OTR value. A direct correlation between OTR and $Y_{X/S}$ was observed and this relationship was inverse for $Y_{P/S}$ at OTR values higher than 5 mmol/L/h. From the data summarized in Table 4, it can be said that the microaeration at OTR= 5 mmol/L/h increased ethanol productivity by promoting sugar uptake ratio and utilization of these sugars for the ethanol pathway compared with that at unaerated conditions. However, the aeration at OTR= 16 mmol/L/h resulted in the lowest ethanol concentration due to ethanol consumption by the cells as well as the lowered specific ethanol production, even though the sugar uptake ratio increased compared to that observed at OTR= 0 mmol/L/h. These findings were in agreement with those of Khongsay et al. [22].

The mass transfer of oxygen molecules from the gaseous phases into the culture fluid is a purely physical process and is described with the oxygen mass transfer coefficient (k_La). In this study, as the aeration rate was increased, the k_La value was also increased (Table 4). In a bioreactor, k_La increases with increasing the bubble diameter due to the changes in hydrodynamics. A decrease in surface tension in presence of alcohol in the reactor results in the formation of small bubbles, leading to larger interfacial areas [6]. Volumetric gassed power consumption (P_g/V_L) decreases depending on the aeration rate in a bioreactor, contrary to k_La and superficial gas velocity. In this study, increasing the aeration rate at constant agitation speed reduced the P_g/V_L value (Table 4).

The dissolved oxygen concentration in a bioreactor depends on the rate of oxygen transfer from the gas phase to the liquid, on the rate at which oxygen is transported into the microorganism, and on the oxygen uptake rate (OUR) by the cells for growth, maintenance and production [23]. Therfore, the OTR value inevitably accompanies with the OUR and oxygen consumption rate. Pinches and Pallent [24] reported that k_La and oxygen uptake rate was related to the rate of cell growth and cell concentration. As shown in Fig 2, the microaerated culture allowed higher viable cell mass and, due to the increase of biomass concentration, the specific ethanol yield was also promoted. It was reported that oxygen had positive effects on ethanol production by increasing the production of biomass and ethanol [25]. Alfenore et al. [26] maximized specific ethanol yield using microaerated conditions by *S. cerevisiae*. However, when values higher than 5 mmol/L/h were applied, the biomass

Accepted Manuscript

ACCEPTED MANUSCRIPT

formation were higher (Fig 2a), this did not lead to an increase in specific ethanol production (Fig 2b) and specific sugar consumption (Fig 2c). Moreover, higher aeration rates resulted in higher cell growth, whereas ethanol production was favored by lower aeration rates. The effect between ethanol production and growth can be explained by energetic relations, which suggested that the energetic requirement for biomass growth is mainly fulfilled by the increase in ethanol production. Alfenore et al. [26] showed that, under microaerated conditions, the biomass and ethanol production were increased, whereas the by-product formation was reduced. Okuda et al. [5] also showed that, after the first 48 h, *E. coli* KO11 stopped both acetate accumulation and sugar utilization, and started to consume the ethanol in the medium, and the cell concentration increased as the ethanol concentration decreased under aeration rates higher than 4 mmol/L/h. *E. coli* KO11 might shift from sugar consumption to ethanol consumption to produce the NADPH needed for cell synthesis. Based on metabolical researches, it was concluded that, in order to maintain cell viability and NADH balance, limited aeration is required for the conversion of sugars to ethanol [5].

Previously, Lawford et al. [27] investigated the effect of oxygen supply (OTR= 8, 24 and 100 mmol/L/h) on ethanol production from a glucose based sugar mixture using ethanologenic recombinant *E. coli*, and reported that all these oxygen supply levels decreased ethanol productivity by diverting carbon to cell mass and CO_2 generation. Our study revealed that the ethanol concentration increased for microaeration at OTR= 5 mmol/L/h compared with that at OTR= 0 mmol/L/h. The results clearly show that, independent from the reactor geometry and size and at the given conditions, the process achieved the higher ethanol concentration at the OTR value of 5 mmol/L/h than at unaerated conditions.

3.2. Effect of aeration duration

In order to determine the appropriate microaerated condition, different aeration durations were studied at the OTR value of 5 mmol/L/h. As shown in Fig. 3, there were considerable differences on the production of ethanol following oxygen feeding into the bioreactor according to statically analyses ($p<0,05$). Compared to the aeration of 8 hours, the final ethanol concentration was 5.42%, 8.44% and 15.65% lower at aeration of 6, 10 and 12 h, respectively.

The biomass concentration increased significantly in the first 8 h of the experiments to approximately 3.62 g/L and slightly increased to 7.0 g/L at 48 h corresponding to 19.66 g/L

Accepted Manuscript

ACCEPTED MANUSCRIPT

ethanol production at OTR value of 5 mmol/L/h for the first 8 h (Fig 4a). When air was supplied for the first 8 hours to the reactor, the cells were able to grow faster and subsequently, when aeration was stopped, higher yields of ethanol were obtained. Joyce [28] reported that the oxygen present earlier in the fermentation broth was rapidly used up for the synthesis of membrane components, which were essential for growth. In an other study, 0.42 g/L ethanol was produced using microaerated conditions of 1 vvm for the first 10 h by *S. cerevisiae var. ellipsoideus* from enzymatic hydrolysate of sunfower hulls [29].

Higher aeration durations lead to decreased specific ethanol productions (Fig 4b) and specific sugar consumptions (Fig 4c). According to Skoog and Hahn-Hägerdal [30], oxygen plays an important role in cell growth, redox balance, functioning of the mitochondria, and generation of energy for sugar transport in *E. coli*. However, excess oxygenation could lead to low ethanol yields, especially in viscous broths. Several studies have shown that ethnaol was produced under anaerobic conditions, but microaerobic conditions for minimal durations appeared to enhance the ethanol production [31, 32].

As seen in Table 5, oxygen enhanced the product yield at lower aeration durations. The maximum ethanol concentration of 19.66 g/L was obtained at the aeration period of 8 h. It was reported that aeration (up to 9 mg O_2/L) during the initial stage of microorganism growth, along with a constant agitation increased the ethanol production from 85.2 to 143.8 g/L (68.7 %) using a synthetic medium containing 305 g/L of glucose by *S. cerevisiae* in 54 h [33]. However as seen in Table 5, an excess of aeration time directly affects micrroorganisms towards the aerobic respiratory pathways for biomass formation, thus a lower fermentation efficiency is obtained.

4. Conclusions

This is the first report on successful utilization of unpretreated quince pomace for bioethanol production in lab scale fermentation using the ethanologenic recombinant *E. coli* KO11. The experiment with an OTR of 5 mmol/L/h for the first 8 hours appeared as optimal for ethanol production by *E. coli* KO11 using microaeration. In this assay, the best results for maximum ethanol concentration (19.66 g/L) and ethanol yield (0.33 g ethanol/g sugars) were attained, which are higher than values reached under unaerated conditions. Limited aeration promoted the sugar uptake ratio compared with anaerobic condition, resulting in a higher ethanol production rate. The further goal of these results is to scale up the process to industrial-scale

Accepted Manuscript

ACCEPTED MANUSCRIPT

production. From the viewpoint of the industrial-scale production of bioethanol, it is a very practical operation to control fermentation performance through OTR, because OTR can theoretically be controlled by adjusting the aeration rate when the reactor shape is known. It was found that microaeration enhances ethanol productivity by promoting sugar utilization. The microaerated fermentation can be considered as a practically applicable process for pilot scale ethanol fermentation. The results obtained in this study will provide valuable guidelines for engineering bioethanol producers.

Acknowledgements

This study was a part of COST Action of the European Union, FP0602, and the authors wish to thank The Scientific and Technical Research Council of Turkey (TUBITAK) for the financial support.

Abbreviations

D	Impeller diameter, m
k	Constant with magnitude dependent on the geometry of the impeller, dimensionless
N	Impeller rotation speed, rpm
N_P	Power number for stirred tank, dimensionless
P	Unaerated power consumption for stirred tank, W
P_g	Aerated power consumption for stirred tank, W
Re	Impeller Reynolds number, dimensionless
V_L	Working volume of the stirred tank, m^3
X	Biomass concentration, g/L
t_m	Mixing time, s
v_{tip}	Impeller tip speed, m/s
k_La	Oxygen mass transfer coefficient, 1/s
q_{O_2}	Specific oxygen uptake rate, mmol/g/h
C^*	Dissolved oxygen concentration at saturation in the bulk, g/m^3
C_L	Dissolved oxygen concentration at any time, g/m^3
OTR	Oxygen transfer rate in the reactor, $mmol/m^3/h$
$Y_{P/S}$	Product yield, g/g

Accepted Manuscript

ACCEPTED MANUSCRIPT

Q_P	Volumetric productivity, g/L/h
v_s	Superficial gas velocity, m/s
Q	Air flow rate, m^3/s

Greek symbols

μ_{max}	Maximum specific growth rate, 1/h
λ	Kolmogorov's eddy size, m
γ	Shear rate, 1/s
τ	Shear stress, N/m^2
ρ	Fluid density, kg/m^3
η	Dynamic viscosity of fluid, kg/m/s
υ	Kinematic viscosity of fluid, m^2/s

References

[1] G. Huerta-Beristain, J. Utrilla, G. Hernandez-Chavez, F. Bolívar, G. Gosset, A. Martinez, Specific ethanol production rate in ethanologenic *Escherichia coli* strain KO11 is limited by pyruvate decarboxylase, J Molecular Microbiol. and Biotechnol., 15 (2008) 55-64.

[2] M. Yasuda, A. Miura, T. Shiragami, J. Matsumoto, I. Kamei, Y. Ishii, K. Ohta, Ethanol production from non-pretreated napiergrass through a simultaneous saccharification and fermentation process followed by a pentose fermentation with *Escherichia coli* KO11, Journal of Bioscience and Bioengineering, (2012) 114(2): 188-192.

[3] B. Cemeroğlu, F. Karadeniz, M. Özkan, Fruit-Vegetable Process Technologies-II, Food Technologies Publications (Turkish), Turkey, 2001.

[4] L. Ingram, P. Gomez, X. Lai, M. Moniruzzaman, B. Wood, L. Yomano, S. York, Metabolic engineering of bacteria for ethanol production, Biotechnology and Bioengineering, 58 (1998) 204-214.

[5] N. Okuda, K. Ninomiya, M. Takao, Y. Katakura, S. Shioya, Microaeration enhances productivity of bioethanol from hydrolysate of waste house wood using ethanologenic *Escherichia coli* KO11, Journal of Bioscience and Bioengineering, 103 (2007) 350-357.

[6] F. Vardar-Sukan, Dynamics of oxygen mass transfer in bioreactors. I: Operating variables affecting mass transfer, Process Biochemistry, 20 (1985) 181-184.

[7] Y. Hang, C. Lee, E. Woodams, A solid state fermentation system for production of ethanol from apple pomace, Journal of Food Science, 47 (1982) 1851-1852.

[8] M. Ngadi, L. Correia, Kinetics of solid-state ethanol fermentation from apple pomace, Journal of Food Engineering, 17 (1992) 97-116.

[9] Y. Hang, Production of fuels and chemicals from apple pomace, Food Technology, 41 (1987).

[10] A. Walkley, I.A. Black, An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method, Soil Science, 37 (1934) 29-38.

Accepted Manuscript

ACCEPTED MANUSCRIPT

[11] D. Barbano, R. Rasmussen, J. Lynch, Influence of milk somatic cell count and milk age on cheese yield, J. of Dairy Sci., 74 (1991) 369-388.
[12] J. Nielsen, J. Villadsen, Bioreactors: Description and modelling-Biotechnology Set, second edition, New York, 1993.
[13] K. Van t Riet, J. Tramper, Mixing, in: Basic Bioreactor Design, Taylor & Francis Inc., New York, 1991, pp. 183-220.
[14] C.J. Geankoplis, Transport processes: Momentum, Heat and Mass, Ohio State Univ., 1983.
[15] P.M. Doran, Bioprocess Engineering Principles, Elsevier Academic Press, New York, 1995.
[16] J.E. Bailey, D.F. Ollis, Biochemical Engineering Fundamentals, second edition, McGraw-Hill Chemical Engineering Series, New York, 1986.
[17] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chemistry, 31 (1959) 426-428.
[18] N. Azbar, F. Tutuk, T. Keskin, Effect of organic loading rate on the performance of an up-flow anaerobic sludge blanket reactor treating olive mill effluent, Biotechnol. and Bioprocess Engineering, 14 (2009) 99-104.
[19] F.K. Agbogbo, G. Coward-Kelly, M. Torry-Smith, K. Wenger, T.W. Jeffries, The effect of initial cell concentration on xylose fermentation by *Pichia stipitis*, Appl. Biochemistry and Biotechnology, 137 (2007) 653-662.
[20] C. Bellido, G. González-Benito, M. Coca, S. Lucas, M.T. García-Cubero, Influence of aeration on bioethanol production from ozonized wheat straw hydrolysates using *Pichia stipitis*, Bioresource Technology, 133 (2013) 51-58.
[21] T.H. Lin, C.F. Huang, G.L. Guo, W.S. Hwang, S.L. Huang, Pilot-scale ethanol production from rice straw hydrolysates using xylose-fermenting *Pichia stipitis*, Bioresource Technology, 116 (2012) 314-319.
[22] N. Khongsay, L. Laopaiboon, P. Jaisil, P. Laopaiboon, Optimization of agitation and aeration for very high gravity ethanol fermentation from sweet sorghum juice by *Saccharomyces cerevisiae* using an orthogonal array design, Energies, 5 (2012) 561-576.
[23] F. Vardar-Sukan, Dynamics of oxygen mass transfer in bioreactors. II: Design variables, Process Biochemistry, 21 (1986) 40-44.
[24] A. Pinches, L.J. Pallent, Rate and yield relationships in the production of xanthan gum by batch fermentations using complex and chemically defined growth media, Biotechnology and Bioengineering, 28 (1986) 1484-1496.
[25] R. Luedeking, E.L. Piret, Transient and steady states in continuous fermentaion. Theory and experiment, J. of Biochemical and Microbiological Technol. and Engineering, 1 (1959) 431-459.
[26] S. Alfenore, X. Cameleyre, L. Benbadis, C. Bideaux, J.L. Uribelarrea, G. Goma, C. Molina-Jouve, S. Guillouet, Aeration strategy: a need for very high ethanol performance in *Saccharomyces cerevisiae* fed-batch process, Appl. Microbiol. and Biotechnol., 63 (2004) 537-542.
[27] H.G. Lawford, J.D. Rousseau, Relative rates of sugar utilization by an ethanologenic recombinant *Escherichia coli* using mixtures of glucose, mannose, and xylose, Appl. Biochemistry and Biotechnol., 45 (1994) 367-381.
[28] G. Joyce, R. Hammond, D.C. White, Changes in membrane lipid composition in exponentially growing *Staphylococcus aureus* during the shift from 37 to 25 °C, J. of Bacteriology, 104 (1970) 323-330.

ACCEPTED MANUSCRIPT

[29] S.K. Sharma, K.L. Kalra, H.S. Grewal, Fermentation of enzymatically saccharified sunflower stalks for ethanol production and its scale up, Bioresource Technology, 85 (2002) 31-33.
[30] K. Skoog, B. Hahn-Hägerdal, Effect of oxygenation on xylose fermentation by *Pichia stipitis*, Appl. and Environ. Microbiology, 56 (1990) 3389-3394.
[31] F.K. Agbogbo, G. Coward-Kelly, Cellulosic ethanol production using the naturally occurring xylose-fermenting yeast, *Pichia stipitis*, Biotechnology Letters, 30 (2008) 1515-1524.
[32] J.P.A. Silva, S.I. Mussatto, I.C. Roberto, The influence of initial xylose concentration, agitation, and aeration on ethanol production by *Pichia stipitis* from rice straw hemicellulosic hydrolysate, Appl. Biochemistry and Biotechnology, 162 (2010) 1306-1315.
[33] Y. Liu, T. Qi, N. Shen, M. Gan, Y. Jin, H. Zhao, Improvement of ethanol concentration and yield by initial aeration and agitation culture in very high gravity fermentation, Yingyong Yu Huanjing Shengwu Xuebao, 15 (2009) 563-567.

Figure Captions

Fig 1. Time profiles of ethanol fermentation by *E. coli* KO11 under microaerated condition at various OTRs of 0, 2, 5, 9, 16 mmol/L/h for the first 8 hours. Fermentations were conducted using a 5 L stirred tank reactor at 35°C, pH 5.5 for 64 h.

Fig 2. Time profiles of (a) biomass concentration, (b) specific ethanol production and (c) specific sugar consumption by *E. coli* KO11 under microaerated condition at various OTRs of 0, 2, 5, 9, 16 mmol/L/h for the first 8 hours. Fermentations were conducted using a 5 L stirred tank reactor at 35°C, pH 5.5 for 64 h.

Fig 3. Time profiles of ethanol fermentation by *E. coli* KO11 under microaerated condition for various aeration durations of 6, 8, 10, 12 hours at OTR value of 5 mmol/L/h. Fermentations were conducted using a 5 L stirred tank reactor at 35°C, pH 5.5 for 64 h.

Fig 4. Time profiles of (a) biomass concentration, (b) specific ethanol production and (c) specific sugar consumption by *E. coli* KO11 under microaerated condition at various aeration durations of 6, 8, 10, 12 hours (OTR= 5 mmol/L/h). Fermentations were conducted using a 5 L stirred tank reactor at 35 °C, pH 5.5 for 64 h.

Accepted Manuscript

ACCEPTED MANUSCRIPT

Table 1. Elementel analysis and sugar composition of dried quince pomace.

The elemental composition	**(%)**	**Sugar***	**(%)**
C	34.50	Glucose	28.76
N	0.23	Fructose	55.71
C/N		Sucrose	10.12

* [3]

Accepted Manuscript

ACCEPTED MANUSCRIPT

Table 2. Equations for the determination of the rheological conditions and hydrodynamic parameters in the reactor.

Mathematical equations			
$Re = \frac{\rho N D^2}{\eta}$	(8)	$\gamma = k N$	(12)
$N_p = \frac{P}{\rho N^3 D^5}$	(9)	$\eta = \frac{\tau}{\gamma}$	(13)
$P_g = 6.35 \left(\frac{P^2 N D^3}{Q^{0.08}}\right)^{0.39}$	(10)	$\lambda = \left(\frac{v^3}{N_p N^3 D^2}\right)^{0.25}$	(14)
$v_s = \left[\frac{k_L a}{0.026 X \left(\frac{P_g}{V_L}\right)^{0.4}}\right]^2$	(11)		

Accepted Manuscript

ACCEPTED MANUSCRIPT

Table 3. Summary of the rheological behaviors of the fermentation broth.

Property	
Re	13187.23
N (rpm)	300
υ_{tip} (m/s)	0.94
γ (1/s)	3000
τ (N/m^2)	4.23
N_P	5.20
P (W)	0.52
P/V (W/m^3)	261.06
ρ (kg/m^3)	1033
λ (m)	3.23x10^{-5}
η (kg/m/s)	1.41 x10^{-3}
υ (m^2/s)	1.36 x10^{-6}

Accepted Manuscript

ACCEPTED MANUSCRIPT

Table 4. Kinetic and hydrodynamic parameters of the fermentation broth at different aeration rates for bioethanol production.

	Aeration rate (vvm)				
	0	0.02	0.035	0.047	0.062
Ethanol (g/L)	17.15	18.47	19.66	16.35	11.24
$Y_{P/S}$ (g/g)	0.29	0.31	0.33	0.27	0.19
$Y_{X/S}$ (g/g)	0.1	0.12	0.12	0.13	0.14
μ_{max} (1/h)	0.02	0.03	0.04	0.06	0.02
Overall ethanol yield (%)	56.05	60.36	64.25	53.43	36.73
Sugar uptake ratio (%)	75.18	92.01	93.49	91.76	91.80
k_La (1/s)	0	0.008	0.012	0.017	0.027
P_g/V_L (W/m^3)	-	0.30	0.26	0.24	0.22
v_s (m/s)	-	0.25	0.62	1.33	3.65
OTR (mmol/L/h)	0	2	5	9	16

Accepted Manuscript

ACCEPTED MANUSCRIPT

Table 5. Kinetic parameters for the bioethanol production at different aeration durations.

	Aeration duration (h)			
	6	8	10	12
Ethanol (g/L)	19.01	19.66	18.13	17
$Y_{P/S}$ (g/g)	0.32	0.33	0.30	0.28
$Y_{X/S}$ (g/g)	0.11	0.12	0.13	0.13
μ_{max} (1/h)	0.02	0.03	0.03	0.04
Overall ethanol yield (%)	62.12	64.25	59.25	55.56
Sugar uptake ratio (%)	85.48	93.49	92.50	92.04

Accepted Manuscript

ACCEPTED MANUSCRIPT

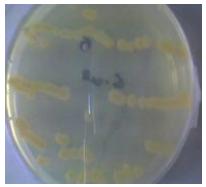
Highlights

- Bioethanol production by *E. coli* KO11 was studied under microaerated conditions.
- Oxygen feeding for the first 8 h at 5 mmol/L/h maximized the ethanol concentration.
- The sugar consumption rate was promoted under aerated fermentation.
- Higher specific growth rate was obtained at aerated conditions.
- 14.44% higher ethanol production was achieved using aeration.

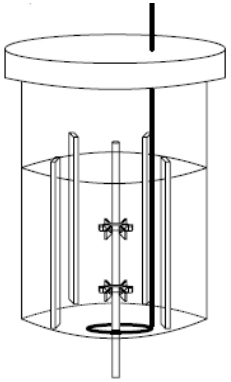
Accepted Manuscript

Graphical Abstract (for review)

Escherichia coli
KO11



Quince pomace +
Luria Bertani Broth



Microaerated stirred tank reactor
0.035 vvm for the first 8 h

19.66 g/L ethanol
(14.44% higher than unaerated reactor)

93.49% sugar uptake ratio
(24.24% higher than unaerated reactor)

ACCEPTED MANUSCRIPT

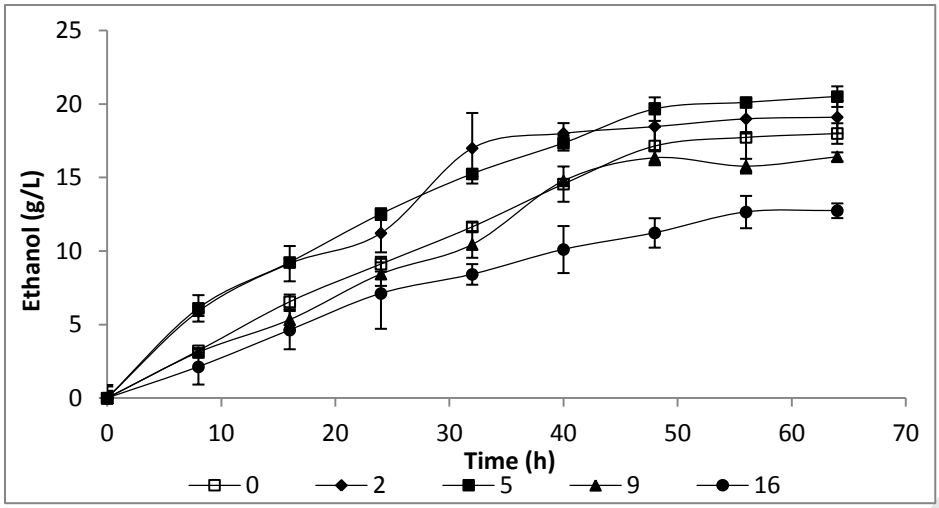


Fig 1.

Accepted Manuscript

ACCEPTED MANUSCRIPT

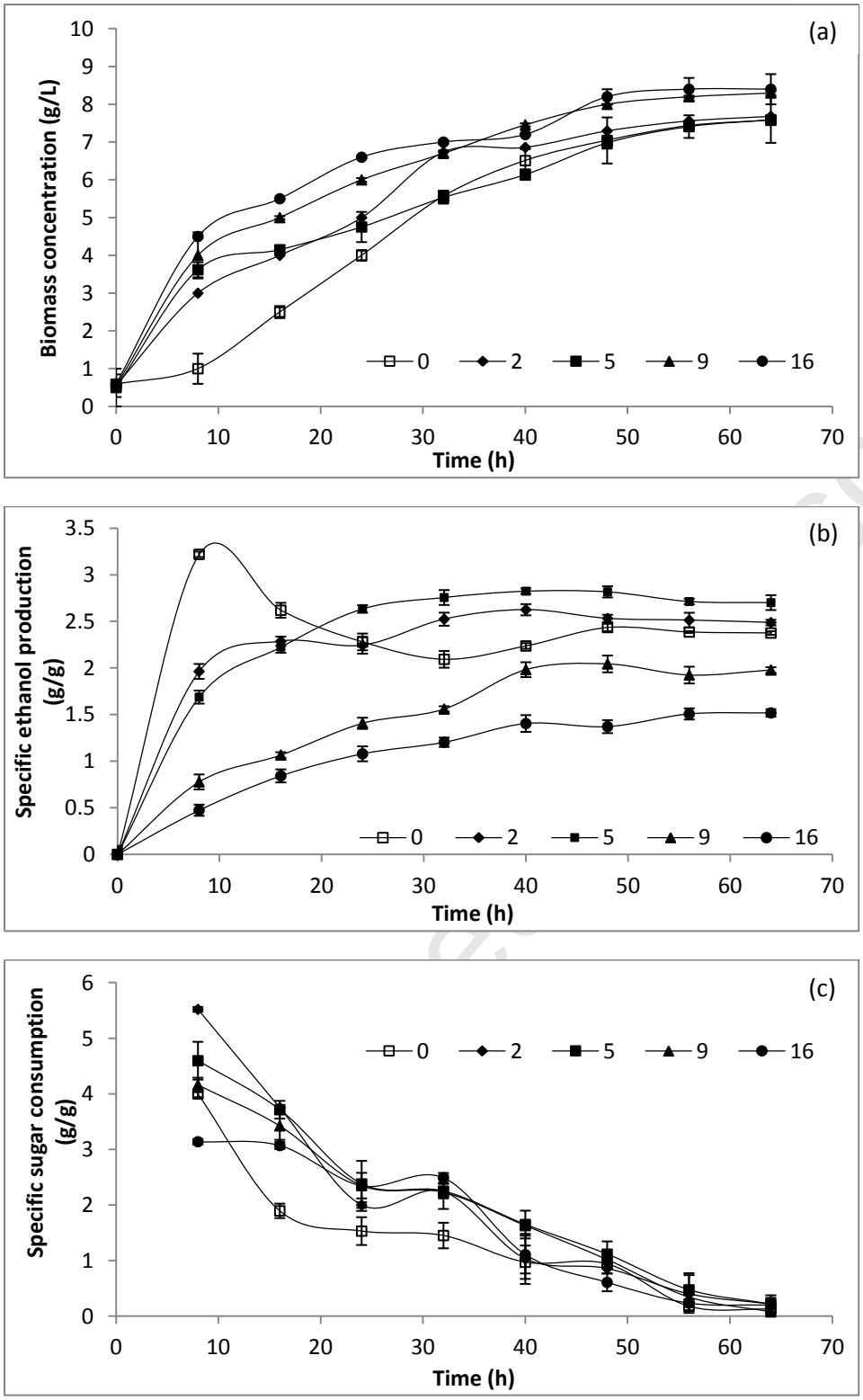


Fig 2.

ACCEPTED MANUSCRIPT

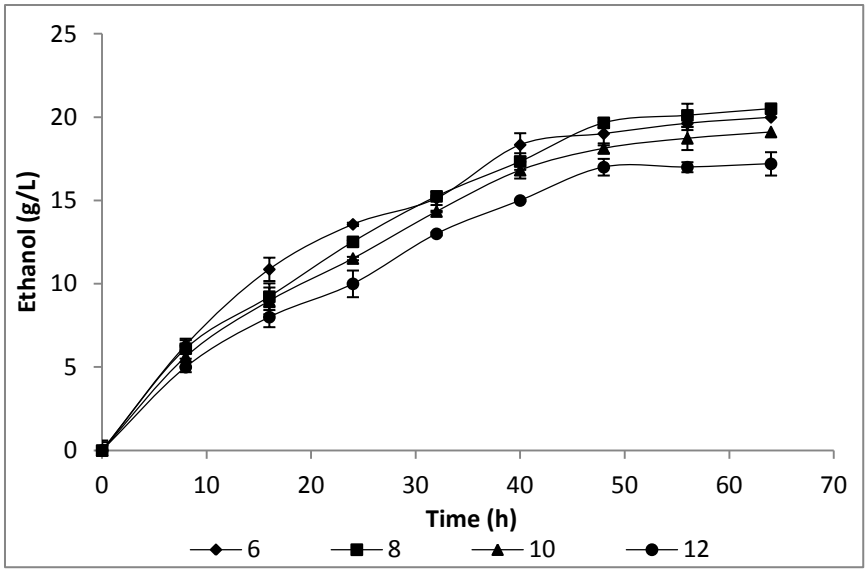
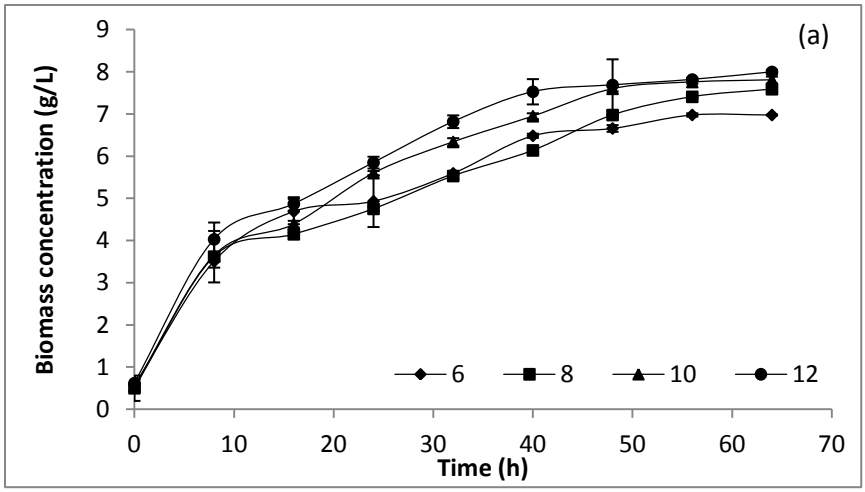
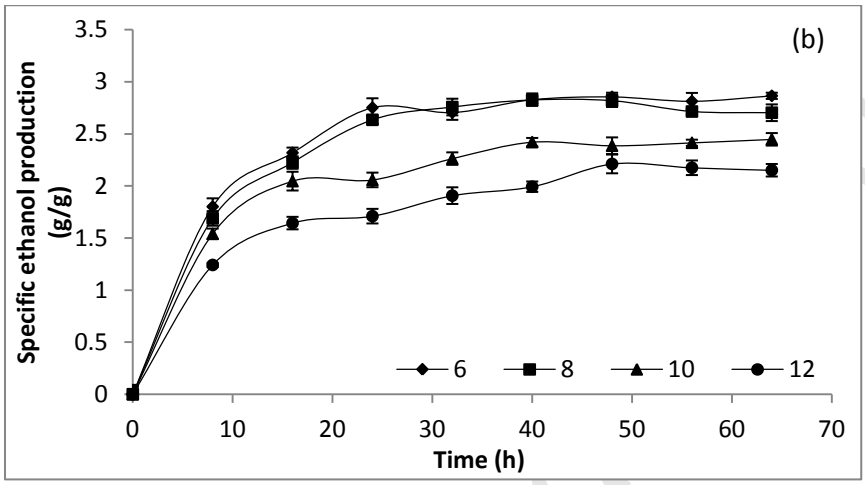


Fig 3.

Accepted Manuscript

ACCEPTED MANUSCRIPT





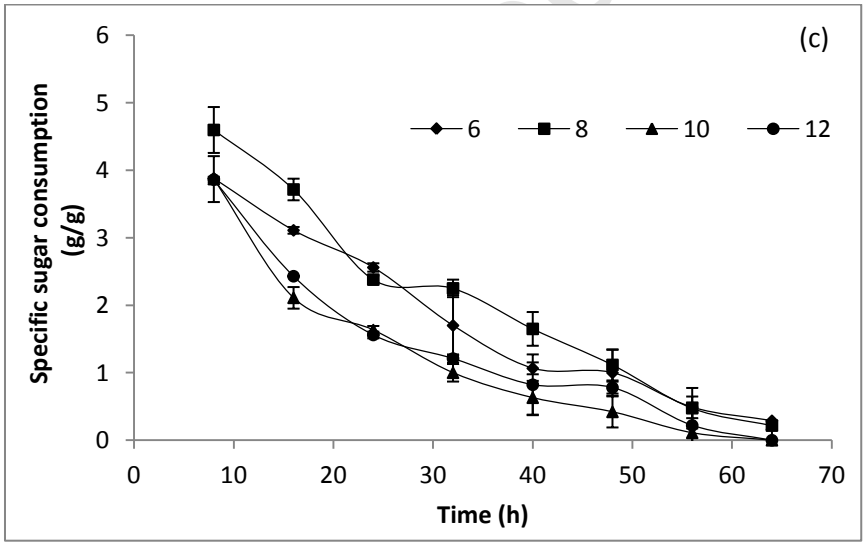


Fig 4.