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Fermentação Primária da Cerveja em Contínuo - Cinética de Imobilização da Levedura e Qualidade do Produto

Continuous Primary Fermentation of Beer - Yeast Immobilization Kinetics and Product Quality

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SUMMARY

A one stage continuous primary beer fermentation consisting of brewing yeast immobilized on spent grain particles in a gas lift reactor was studied. The goal of this work was to adjust the flavor of the continuously produced green beer to the desired character by sparging an adequate amount of air and by controlling the fermentation temperature in the immobilized yeast reactor as well as to predict the rate of the brewing yeast immobilization using a kinetic model adapted to the conditions of beer fermentation. The volumetric productivity of the continuous system was approximately 5 times higher than of the batch fermentation. The aroma profile of green beer from the continuous immobilized fermentation, run at zero air flow and temperatures 13 16°C, was fully comparable to that produced by industrial batch technology. Generally, the diacetyl concentration in green beer from the continuous fermentation was higher than in batch process, however, its re-assimilation was enhanced by high total biomass concentration in the system.

SUMÁRIO

Foi estudada a fermentação primária em contínuo da cerveja num reactor "gas-lift" com a levedura imobilizada em "drêches". O objectivo do trabalho foi optimizar as características organolépticas da cerveja verde produzida em contínuo através do controle do caudal e composição do ar e da temperatura. Pretendeu-se também prever a velocidade de imobilização de levedura cervejeira sendo, para tal, desenvolvido um modelo adaptado às condições de fermentação.

A produtividade volumétrica do sistema contínuo foi cerca de 5 vezes superior à obtida em descontínuo. O perfil aromático da cerveja verde produzida em contínuo, utilizando um caudal nulo de ar e temperaturas entre os 13 e os 16º C, foi comparável ao perfil aromático da cerveja produzida em descontínuo, numa unidade industrial.. Na generalidade das situações testadas, a concentração de diacetilo na cerveja verde obtida em contínuo foi maior que no processo descontínuo sendo a sua reassimilação aumentada pela maior concentração de biomassa no sistema contínuo.

PALAVRAS-CHAVE
KEY WORDS

Cerveja, contínuo, fermentação, imobilização, "drêches", modelo cinético
Beer, continuous, fermentation, immobilization, spent grains, kinetic model

1. INTRODUCTION

Today the brewing industry applies a broad spectrum of novel engineering, biochemical, microbiological and genetic inventions. Thanks to these contemporary achievements this traditional industry became similar to those referred to as "new biotechnologies" (PILKINGTON et al. 1998). Nevertheless, some of the new possibilities, e.g. continuous beer fermentation with immobilized brewing yeast, have still not been intensely commercialized. The reason for frequent marginalization of continuous brewing lies in the often legitimate objections of the industry towards technical difficulties accompanying the process as well as in the desire of the brewers to preserve the traditional image approved by the consumer (MENSOUR et al 1997).

Despite of the advantages that continuous beer fermentation offers, mostly of economic origin, the technical difficulties such as demanding process control, flavour problems, risk of contamination, yeast viability, carrier price and the inconvenience of immobilization retard the implementation of the process at industrial scale (LINKO et al. 1998). However, cheap carrier materials applied in suitable reactor configuration could inspire researchers and encourage brewing engineers to consider the industrial application of this process.

The goal of this paper is to describe the use of spent grain particles as a carrier for brewing yeast immobilization and its application in continuous beer fermentation in a gas-lift bioreactor. Special attention will be paid to the optimization of operational conditions (aeration and temperature) in terms of volumetric productivity and sensorial quality of the beer after primary fermentation (green beer). Another aspect that will be addressed is the adjustment of a simple kinetic model describing the immobilization rate of the brewing yeast to spent grain particles during real beer fermentation.

2. METHODOLOGY

2.1 Yeast strain and culture conditions

The brewing yeast Saccharomyces uvarum (carsbergensis) was supplied by the brewing company UNICER, SA. The yeast for inoculation of the continuous airlift reactor were cultivated in 500 mL of synthetic medium under aerobic conditions on a rotary shaker (120 rpm) at 30ºC for 30 h. The composition of the synthetic medium was as follows (g/L): KH2PO4, 5.0; (NH4)2SO4, 2.0; MgSO4.7H2O, 0.4; yeast extract, 1.0; glucose, 10.0. Medium with the same composition was used in continuous experiments during biomass attachment. The all malt wort used in this work had an original gravity of 14ºP and was supplied by UNICER, SA.

2.2 Carrier preparation

Dry spent grains were mixed in 3 vol % HCl to hydrolyse the residual starchy endosperm and embryo of the barley kernel present in the spent grains. Then the mixture was washed with water and dried. The remaining solids mainly the husks of the barley grain were partially delignified by shaking in 2 % (wt/vol) NaOH. After being washed several times with water (until neutral pH) and dried, the carrier was ready to be used. For more detailed description see BRáNYIK et al. 2001.

2.3 Gas-lift reactor (GLR)

The GLR used in this work (Fig.1) is of the concentric draught tube type with an enlarged top section for degassing and a total working volume of 6 L. The dimensions of the reactor can be found in BRáNYIK et al. 2002. The temperature inside the reactor (10 - 16°C) was maintained by means of a cooling coil connected to a refrigeration bath. Air flow rate was adjusted using a mass flow controller (Hastings 202D, Hastings Instruments, USA) while CO2 flow rate was regulated by a rotameter.

2.4 Starting and operating of GLR

The Plexiglas GLR reactor was sterilized using sodium hypochlorite solution (2 % active chlorine) at least 4 days prior to fermentation. After draining the reactor the sterile gas supply was started at a total flow rate (mixture of air and CO2) of 0.25 L/min and the reactor was filled with the sterilized slurry consisting of spent grains (80 g dry state) in distilled water (3 L). Prior to inoculation, the reactor containing fresh carrier was washed with 50 L of sterile water. Subsequently, the reactor was charged with concentrated medium to obtain the desired concentration of the synthetic medium and then inoculated with 2×500 mL of yeast cell suspension grown using a rotary shaker. At the end of 24 h batch growth, synthetic medium started being fed at a dilution rate (D) of 0.16 h-1, which was after 168 h of operation increased to 0.25 h-1. At 225 h, the synthetic medium was changed to sterilized wort (50 L, 40 min at 120ºC), which was used throughout the whole fermentation experiment at D = 0.05 h-1. In order to prevent oxidation, wort was during the whole experiment kept in a refrigeration unit at 6 - 8°C under N2 atmosphere. During wort fermentation the total gas flow rate in the reactor (mixture of air and CO2) was kept constantly at 0.25 L/min, with different proportions of air in the mixture. The continuous system was considered to be in steady state conditions after a period of 5 residence times.

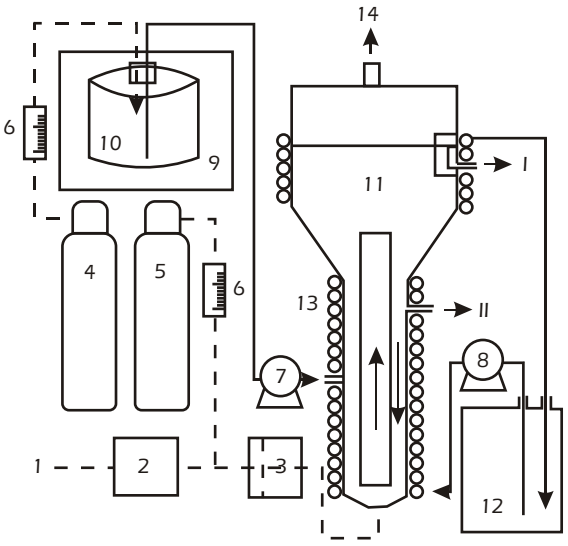

Figure 1. Laboratory scale installation for primary beer fermentation: 1-air supply; 2-mass flow controller; 3-gas sterilization filter; 4-N2 bottle; 5-CO2 bottle; 6-rotameter; 7-peristaltic pump; 8-centrifuge pump; 9-refrigeration unit; 10-wort barrel; 11-gas-lift reactor; 12-refrigeration bath; 13-cooling coil; 14-gas outlet; I.-green beer sampling point; II.-carrier sampling point.

2.4 Analytical and computational methods

Characterization of wort, green beer and beer (specific gravity, original extract, degree of attenuation, alcohol, pH, and colour) was performed by SCABA 5600 (Automatic Beer Analyser, Tecator AB, Sweden). Total Diacetyl was determined by gas chromatographic analysis of the static headspace (VAN IERSEL et al. 1999). The flavour and aroma compounds (higher alcohols and esters) were measured according to the current European Brewery Convention recommended methods. The detailed procedure of the immobilized biomass (Xim) determination can be found in BRáNYIK et al. 2004a. The kinetic model was integrated in the computer program *PSI V2.00a* (Boza Automatisering BV, Nuenen, The Netherlands).

3. RESULTS AND DISCUSSION

3.1 Immobilization of brewing yeast to spent grain particles

The immobilization of yeast onto spent grains in the gas-lift reactor (GLR) started by feeding the reactor with synthetic medium. The start-up period of the reactor, time necessary to build up approximately 0.3 gIB/gC (IB dry immobilized biomass, C dry carrier) immobilized biomass (Xim), was characterized by high media consumption. Therefore, the choice of the synthetic medium helped us to avoid difficulties with supply and storage of wort. The start-up period of the continuous experiments in the GLR when synthetic medium was used as a feed, initially at D = 0.16 h-1 and then D = 0.25 h-1, gave rise to the spontaneous attachment of the brewing yeast to the surface of the spent grain particles (Fig. 2). When Xim reached 0.3 gIB/gC, the reactor feed was changed to wort (at 225 h), D was lowered to 0.05 h-1 and the air supply was lowered from 0.25 L/min to 0.05 L/min. The significantly higher sugar concentration in wort (ca. 110 g/L fermentable sugars) further increased the amount of immobilized biomass (ca. 0.5 gIB/gC). Simultaneously, the change to a fermentation medium with higher sugar concentration (i.e. wort) resulted in increased free biomass concentration (Fig. 2). Although the start-up period was carried out with synthetic medium as feed, experiments in bubble-column reactor (BCR) illustrated that the attachment of brewing yeast onto spent grains occurred in brewery wort as well as in the fermentation medium (BRáNYIK et al. 2002).

3.2 Modeling of the yeast immobilization

A kinetic model for simulation of brewing yeast immobilization on spent grain particles and free cell growth in a continuous bubble-column reactor (BCR) fed by synthetic medium has been developed (BRáNYIK et al. 2004b). The model is based on the finite replicative lifespan of the eukaryotic brewing yeast (POWELL et al. 2000), which is expressed in the model through the concept of the active fraction of immobilized biomass ().

Although the parameters of the model were determined for yeast immobilization in BCR, the model gave a reasonable prediction of the dynamics of immobilized biomass accumulation and free cell growth in GLR during start-up period on synthetic medium (Fig. 2). The complete list of the parameters used in the simulation can be found in BRáNYIK et al. 2004b. Only the yield coefficient was found slightly higher in GLR, probably as a result of a better oxygen transfer in this reactor. The agreement of the model simulation with experimental data shows that the hydrodynamic conditions in pneumatically agitated reactors (GLR and BCR) are close insofar, that the model and its parameters can be considered valid in both systems.

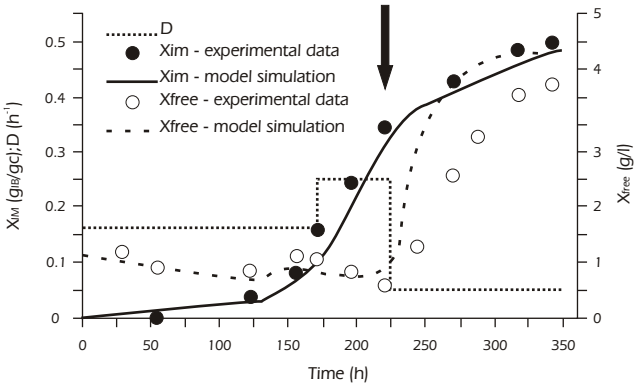

Figure 2. Brewing yeast immobilization to spent grain particles (Xim) and free biomass (Xfree) in continuous GLR. Changes at 225 h of the experiment, as indicated by the arrow, include: change from synthetic medium to wort; decrease of D from 0.25 h-1 to 0.05 h-1; change of the inlet gas composition from 0.25 L/min air to a mixture of 0.05 L/min air and 0.2 L/min CO2; decrease of temperature from 25°C to 16°C.

Nevertheless, in order to predict the immobilized and free biomass balances during the continuous fermentation of wort in GLR, some of the parameters determined in BCR and synthetic medium had to be adjusted to the conditions of beer fermentation (Tab. I.). The lower temperature results mainly in a lower maximum specific growth rate, which subsequently decreases the detachment rate coefficient through the decreased contribution of outgrowth. Another manifestation of the lower growth rate is the deceleration of the yeast aging which can be expressed by an increase in the amount of active biofilm. For the purpose of model simulation the detachment rate coefficient was lowered and the active biofilm concentration was increased by approximately 40 %, corresponding to the maximum growth rate decrease due to temperature drop. The lower aeration rate increased the dominance of the fermentative metabolism as expressed by the lower yield coefficient (Tab. I.). The slight underestimation of Xim and overestimation of Xfree during beer fermentation by

the model (Fig. 2) can be ascribed to inaccurate assessment of some model parameters for wort fermentation.

Table 01. List of model parameters (description, values and units) that change when feed is switched from synthetic medium to wort.

Parameter	Description	Start-up perioda	Beer fermentationb
		Value/Units	Value/Units
X_{im}^{act}	Active biofilm concentration	0.12c gIB/gC	0.2* gIB/gC
$u_{im/free}^{max}$	Maximum specific growth rate	0.29 h-1	0.17 h-1
k_{det}^{sst}	Detachment rate coefficient	0.04c h-1	0.03* h-1
$Y_{X/S}^{im/free}$	Yield coefficient	0.11	0.05
S_o	Influent sugar concentration	10 g/L	110 g/L

[a] Synthetic medium, air flow 0.25 L/min, 25°C.

[b] Wort, air flow 0.05 L/min, 16°C.

[c] Parameters determined in BCR, on synthetic medium at 25°C (BRáNYIK et al. 2004b).

[*] Estimated values.

3.2 Productivity of the continuous beer fermentation

The degree of attenuation shows what percentage of the extract in the wort has been fermented. During primary beer fermentation the attenuation is controlled by the amount of biomass in the reactor (Xtot) and by its metabolic activity. Wort with an original extract of 14oP was, during primary fermentation, converted to green beer in the one stage GLR system to a degree corresponding to values of real attenuation between 38 and 63 % (BRáNYIK et al. 2004c). To what extents are the wort sugars converted to alcohol and other yeast by-products is largely determined by the total biomass concentration (Xtot). On the other hand, Xtot can be controlled by fermentation conditions such as aeration, temperature, solid load and reactor design.

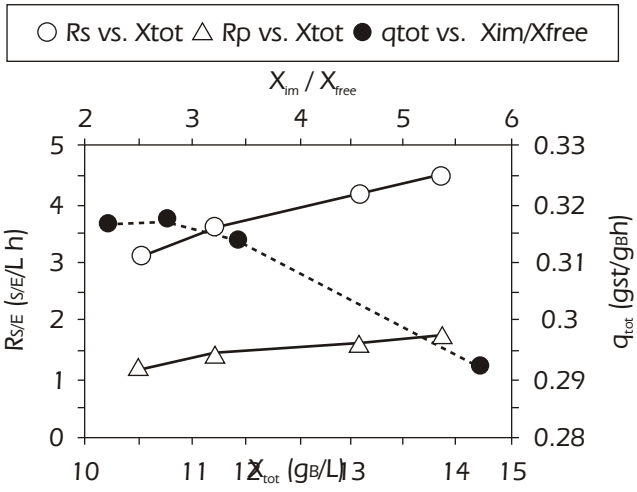

Figure 3. RS - volumetric saccharide consumption rate (gSaccharide/Lh) and RE - volumetric ethanol production rate (gEthanol/Lh) vs. Xtot total biomass concentration in GLR (gBiomass/L) and qtot total specific sugar consumption rate (gSaccharide/gBiomassh) vs. Xim/Xfree immobilized to free biomass ratio.

The higher air supply and/or higher fermentation temperature induced cell growth, observed as an increasing Xtot, was leading to a more extensive wort saccharide consumption and ethanol production. This can be expressed as the increasing rate of saccharide consumption (RS) and ethanol production (RE) per unit reactor volume. Both volumetric productivities (RS and RE) showed values (Fig. 3) similar to those found for a one stage gas-lift bioreactor with yeast immobilized in pectate gel (ŠMOGROVIèOVá et al. 1997) and for a two stage fluidized bed system with glass beads as carrier (TATA et al. 1999). The maximum RS in the ALR system with yeast immobilized on spent grains (ca. 4.2 g/Lh) was significantly higher than the average RS in batch fermentation (ca. 0.8 g/Lh) considering a 14oP wort attenuated to 4oP apparent extract within 5 days.

When Xim/Xfree grew from approximately 2 to 6, the increasing amount of immobilized biomass in GLR system was accompanied by a gradual decline of the total specific saccharide consumption rate (Fig. 3). Because the total specific saccharide consumption rate (qtot) is not constant at different Xim/Xfree ratios, therefore the metabolic activity of the free (Xfree) and immobilized (Xim) cells can not be considered equal. The estimated specific saccharide consumption rate of the immobilized (qim) and free cells (qfree) was 0.25±0.08 gS/gIBh and 0.63±0.10 gS/gFBh, respectively (BRáNYIK et al. 2004c). Since the estimated maximum average biofilm thickness is ca. 10 m (BRáNYIK et al. 2004b), it can be assumed, that rather than substrate diffusional limitation into the multilayer yeast biofilm, it is the different physiological condition (e.g. aging) of the immobilized biomass (BAKER and SMART 1996) that is responsible for its lower metabolic activity.

However, in order to evaluate the performance of the continuous GLR system with spent grains as a carrier for green beer production, besides engineering parameters of the system, it is essential to study the sensory compounds and the flavor profile of the produced green beer.

3. 4 Product flavor profiles

The total diacetyl concentration in the green beer produced in GLR with brewing yeast immobilized on spent grains was significantly above its taste threshold of 0.05 mg/L (LINKO et al. 1998) and depended on the operational conditions. Under conditions of increasing aeration, fermentation temperature (Fig. 4) and Xim the diacetyl concentration in green beer decreased, however, its concentration in green beer was still considerably high. Nevertheless, it was possible to reduce the total diacetyl level in green beer below its taste threshold in the final product within 10 days of batch maturation period at 4 °C (BRáNYIK et al. 2004c). High diacetyl formation during continuous primary beer fermentation imposes significant requirements on

maturation. In order to shorten the maturation, several strategies have been developed such as heat treatment of green beer (YAMAUCHI et al. 1995), fermentation with genetically modified brewer's yeast (KRONLöF and LINKO 1992) etc.

Esters and higher alcohols are volatile compounds that impart to a beer its characteristic flavor and aroma profile. The optimum oxygen supply into the continuous immobilized yeast reactor and ideal fermentation temperature are critical process parameters for adequate beer flavor formation. For example, excess oxygen leads to the production of unnecessary yeast biomass, low ester production but excessive acetaldehyde, diacetyl and fusel alcohol formation (BRáNYIK et al. 2004c; OKABE et al. 1992). Similarly to increased oxygen concentration, high temperatures were also reported to increase the amount of fusel alcohols, esters and acetaldehyde in beer (ŠMOGROVIèOVá and DöMéNY 1999) by influencing the rate of yeast growth.

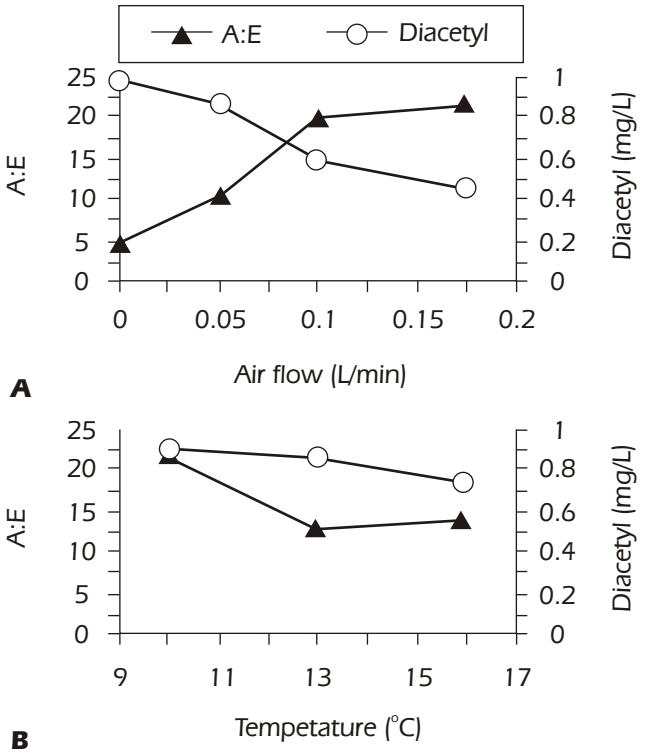

Figure 4. A: Higher alcohols to total esters ratio (A:E) and total diacetyl concentration in green beer vs. air flow rate in GLR at constant temperature 13°C. B: Higher alcohols to total esters ratio (A:E) and total diacetyl concentration in green beer vs. temperature in GLR at constant air flow rate 0.05 L/min.

The optimum higher alcohols to esters ratio (A:E) in lager beers is considered to be between 4:1 and 5:1 (POLEDNíKOVá et al. 1993). An A:E ratio of 4.7 in green beer from the continuous system were achieved at zero air flow (circulation induced by pure CO2) and temperatures 13oC (Fig. 4). Satisfactory volatile ratio (A:E = 4.3:1) was achieved also at zero air flow and 16 °C simultaneously with a relatively low diacetyl content (0.48 mg/L) and a sufficient degree of real attenuation of 54 % (BRáNYIK et al. 2004c).

The aroma profile of the green beer from the continuous immobilized fermentation was compared to a green beer produced by industrial batch technology. For comparison, the values of selected aroma compounds in industrial green beer were set to 100 %. The amount and distribution of aroma compounds in continuously fermented green beer were close to those of a commercial lager beer after primary fermentation (Fig. 5). With the exception of the slightly lower content of higher alcohols, there were no significant differences between the flavor and aroma profiles of the two green beers. Moreover, while the A:E ratio in the lager beer produced by the industrial batch process was slightly higher (5.4:1) than the recommended values for lager beers, in the final beer produced by continuous primary fermentation in GLR and subsequent batch maturation for 10 days at 4°C the A:E was 4.5:1.

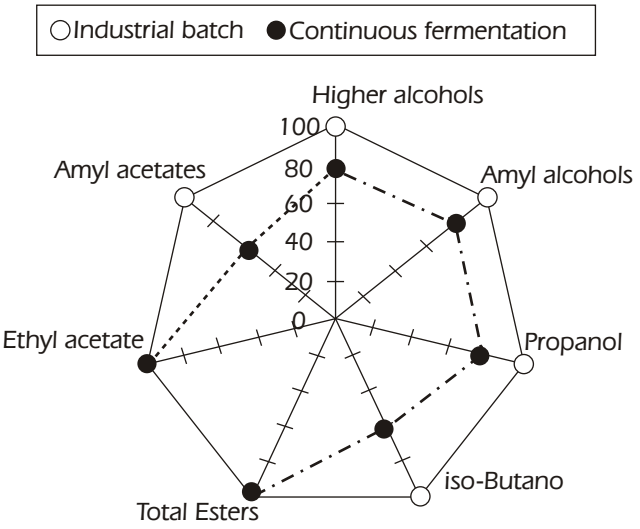

Figure 5. Relative concentrations of selected aroma compounds in green beer after primary fermentation carried out by an industrial batch process and continuous fermentation in ALR at 16 °C and zero air flow.

4. CONCLUSIONS

The recently proposed kinetic model for brewing yeast immobilization on spent grain particles (BRáNYIK et al. 2004b) was applied for the prediction of the dynamics of immobilized biomass accumulation and free cell growth in a GLR during the start-up period on a synthetic medium and during the first 4 days of continuous fermentation of wort. Good agreement between model simulations and experimental data was achieved.

The industrial-scale use of immobilized primary fermentation is dependent on both economic facts and the ability to produce high quality beer. Besides the high volumetric productivity, the presented immobilized cell system

is very promising in terms of process economy. The use of spent grain particles, a brewing by-product, may be considered advantageous, as the carrier cost is a significant part of the equipment cost (VIRKAJäRVI and KRONLöF 1998).

The influence of the operational conditions on the sensorial quality of green beer was also studied. Although the diacetyl formation during continuous primary fermentation was higher than it is usual in industrial batch, it was possible to suppress its formation at low aeration and high total biomass concentration in the GLR. The immobilized yeast system for primary fermentation produced slightly lower levels of total higher alcohols while the amount of total esters was equal to the green beer produced by industrial batch process. The higher alcohols to esters ratio was in the range recommended for lager beers.

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6. ACKNOWLEDGEMENT

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