Research article

Institute of Brewing & Distilling

Received: 7 October 2013 Revised: 4 December 2013 Accepted: 4 December 2013 Published online in Wiley Online Library: 8 January 2014

(wileyonlinelibrary.com) DOI 10.1002/jib.111

Maintaining yeast viability in continuous primary beer fermentation

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Continuous fermentation is a long known and vastly studied process. The use of immobilized cell technology (ICT) is exploited in a significant number of studies owing to the associated high volumetric productivity, time savings and low capital demand. This work was aimed at solving one of the most relevant obstacles to implementing ICT on a large scale in beer fermentations, namely the control of biomass and the maintenance of cell viability in a gas-lift bioreactor. For this purpose, foam fractionation by skimming was proposed as a tool for control of continuous biomass concentration. The consequences of foaming on lignocellulosic yeast carrier losses were assessed and discussed. A steady consumption of sugars from wort, as well as consistent ethanol production, were achieved. The viability of the suspended cells in the reactor was compared with that of the cell population in the foam using flow cytometry. Results suggest that foam might be used as a promising tool to skim non-viable biomass out of the gas-lift reactor, thus ensuring the maintenance of a cell culture with optimum viability.

Keywords: continuous fermentation; lignocellulosic yeast carrier (LCYC); excess of biomass; foam fractionation

Introduction

Without question, the vast majority of fermentation processes are performed in batch mode. However, it is also well known that batch fermentations are time-consuming and their replacement by continuous fermentation processes could lead to important improvements in process productivity and, possibly, to economic gains.

Beer production is one of the processes that has been addressed by several authors concerning the application of continuous fermentation systems *(1–7)*. In traditional batch processes, primary beer fermentation, along with maturation, can take more than a month. Continuous fermentation with immobilized biomass has been proposed as an attractive alternative that may provide the final product in less than a week *(1,2,8,9)*. This high volumetric productivity may lead to savings in capital and operational costs *(10)*. Among the technical issues reported to date as needing improvement *(11,12)*, the maintenance of viable and metabolically active biomass inside the reactor for a prolonged period of time is, undoubtedly, one of the most important. This objective should be achieved while preventing biomass clogging inside the bioreactor *(1,13)*. Moreover, the immobilized biomass must be capable of producing beer that meets the required quality standards *(14)*.

Individual yeast cells have a limited lifespan *(15–17)* and a restricted time of metabolic activity *(18,19)*. For these reasons, in a continuous reactor, dead and injured cells are prone to accumulate with negative consequences on the quality of the final product *(20)*. In the batch process, after primary fermentation, dead cells are removed from the vessel by preferential sedimentation of the older cell population *(21–24)*. In continuous systems where mixing is applied, such as a gas-lift reactor, biomass separation through preferential sedimentation is not feasible. As a consequence, it is fundamental to develop strategies to match the challenge of removing the excess of dead and injured cells without stopping the process in a continuous gas-lift bioreactor.

On the one hand, biofilm formation onto a lignocellulosic yeast carrier (LCYC) is a dynamic process, involving cell deposition, growth of immobilized biomass and detachment *(25)*. On the other hand, aging in brewing yeast cells is followed by changes in the surface properties of the cell wall, with older yeast cells being more hydrophobic then their younger counterparts *(24)*. Hydrophobic particles can be removed from a system by adhering to bubbles and rising to the reactor top, where they are removed by skimming. This process is called foam fractionation *(26–28)*. The hydrophobic character of older cells can thus be used as a strategy for foam fractionation and as a method for the constant removal of aged biomass from a bioreactor.

This work evaluated the effect of foaming on biomass and on the LCYC removal in a continuous beer primary fermentation process, in an air-lift reactor, aiming at an effective control of biomass skimming and at the possibility of differentially skimming non-viable biomass.

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Material and methods

Brewing yeast

The brewing yeast *Saccharomyces carlsbergensis* was generously been supplied by UNICER Bebidas, S.A. (S. Mamede de Infesta, Portugal) from its production line. The yeast was taken from the storage tank for further fermentations, previous to acid treatment. The sample was inoculated on Yeast-Extract Peptone Dextrose broth (Sigma, UK) with 2% agar (Fluka, Switzerland) for yeast colony isolation. A flocculent *Saccharomyces carlsbergensis* yeast, strain 96, from the culture collection of brewing yeast (Research Institute of Brewing and Malting plc., Prague, Czech Republic) was used in a second experiment. Strain 96 was inoculated onto Petri dishes as described above. For both strains, one colony was pitched into 400 mL of wort and incubated at 20°C, and 120 rpm for 48 h, previous to being used as the inoculum for the gas-lift reactor.

Beer wort

Two continuous fermentation trials were performed. In the first trial, wort with an original specific gravity of 15 °P, supplied by UNICER, was used. It was boiled, filled into 50 L autoclavable polypropylene carboys (Nalgene, USA) and autoclaved for 1 h. In the second trial, concentrated wort (Research Institute of Brewing and Malting – RIBM, Prague) was diluted to the desired final concentration, which varied from 5 to 11 °P. Thereafter, it was filled into 20 L autoclavable polypropylene carboys (Nalgene, USA) and autoclaved for 3.5 h.

Brewers' spent grains

Brewers' spent grains were kindly provided by UNICER Bebidas, S.A. (S. Mamede de Infesta, Portugal) from its beer production process. It was double caustic (3 and 6%) treated as previously described *(29)* for LCYC production. After drying, the LCYC was used in both fermentations at 10 g L^{-1} (ratio of 1% dried LCYC/reactor working volume).

Continuous fermentation

All experiments were performed in a Perspex gas-lift reactor with a 4 L total working volume. Gas flow was kept constant (500 mL min^{-1}) using a mass flow controller (GFC17, Aalborg, USA). The temperature was held at 15°C using a Julabo F32 Refrigerated/Heating Circulator (Julabo, Germany) for both fermentations. The wort was fed to the reactor with a peristaltic pump at a constant dilution rate of 0.043 h^{-1}.

The reactor was sterilized using a 3% (v/v) solution of commercial sodium hypochlorite with 1.5% active chlorine, 48 h before use. After this time, the solution was discarded and 50 L of sterile water was used to wash the reactor.

After washing, the reactor was filled with wort and inoculated. It remained under batch operation for 48 h previous to the addition of 40 g of LCYC and the start of the continuous phase. During the batch phase, the supplied gas was pressurized air. This was changed to pure CO_2 at the beginning of the continuous phase. In both cases, the gas was sterilized by passing through a 0.2 μm sterile filter (Whatman, UK). Fig. 1 displays a schematic view of the experimental setup.

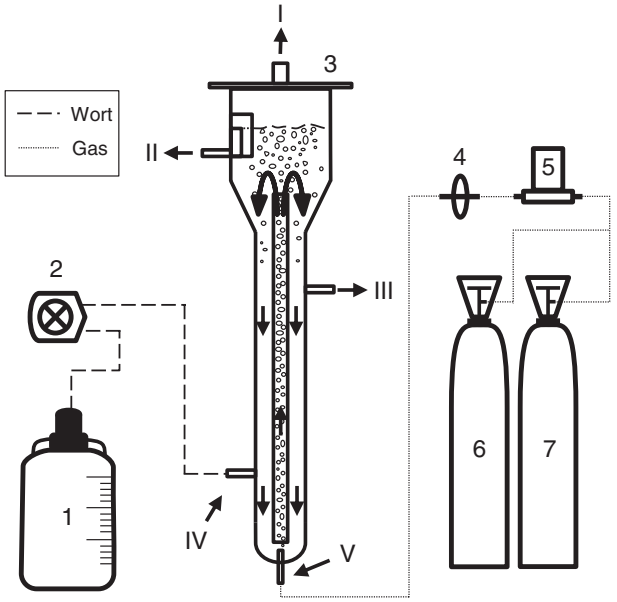

Figure 1. Immobilized yeast reactor system: 1, wort barrel; 2, peristaltic pump; 3, gas-lift reactor; 4, air filter; 5, flow mass controller; 6, pressurized air; 7, CO_2 bottle. I, Gas outflow and foam sampling point; II, green beer outflow/sampling point; III, yeast (biocatalyst, flocculated and free biomass) sampling point; IV, wort inlet; V, gas sparger.

The first fermentation was carried out without the addition of antifoam and the yeast strain used was from UNICER. In the second fermentation, foaming was inhibited by the direct addition into the reactor of a sterile Antifoam B emulsion, containing 10% of active silicone (Sigma, UK), and yeast strain 96 was used.

Biomass measurements

Immobilized biomass was assessed as previously reported *(2)*. Measurements of free biomass were carried out. Three samples of 10 mL from the bulk liquid of the reactor, free of biocatalysts, were collected into pre-weighed 15 mL Falcon tubes and centrifuged at 4000***g*** for 5 min. The liquid phase was discarded and the tubes were dried at 105°C for 24 h and weighed for biomass determination. Controls were samples using the inlet wort to correct for the presence of trub-like proteins, which could interfere at weighing.

Flow cytometric assays

Stock solutions were prepared for fluorescein diacetate (FDA) and propidium iodide (PI). The FDA (Sigma, UK) was diluted with dimethyl sulfoxide (Sigma, UK) to a final concentration of 500 μg mL^{-1}. The PI (Sigma, UK) solution was diluted in ultra-pure water to a final concentration of 1 mg mL^{-1}. The stock solutions were kept at −20°C until used. For the double staining with FDA and PI, 100 μL of ~1×10^6 cell suspension was added to 400 μL of phosphate buffered saline (PBS; Sigma, UK) containing 1 μL of PI stock solution and 4 μL of FDA. Incubation was carried out at room temperature for 20 min.

From the continuous reactor, three types of yeast populations were chosen for flow cytometric analyses: free (not attached to support) flocculated cells, immobilized cells on the LCYC support and cells collected from the foam. Immobilized cell populations were prepared by successive washing of a small amount of

biocatalyst (LCYC plus cells) in PBS followed by a strong agitation for cell release. The cells in the supernatant were used for further characterization. As the biocatalysts had higher density than the flocculated cells, it was easy to separate them by sedimentation. Therefore, after sedimentation, yeast flocks were harvested with the help of a pipette and centrifuged at 4000***g*** for 5 min. The liquid phase was discarded and the cells were washed twice with PBS previous to staining. Samples were double stained with FDA and PI as described above, and analysed using a Partec Pas III (Partec GmbH, Münster, Germany) analyser equipped with an argon ion laser (15 mW laser power with excitation wavelength 488 nm). The FDA green fluorescence was detected by the FL1 (530 nm) channel, while the PI red fluorescence was captured by the FL3 (590–610 nm). More than 20,000 cell readings were carried out for each sample in triplicate. Data were analysed in the Flowing Software version 2.2.0 (Freeware provided by Perttu Terho, Turku Centre for Biotechnology, University of Turku, Finland).

Epifluorescence microscopy

Samples were periodically analysed by epifluorescence microscopy, using an Olympus BX51 (Olympus, Japan) microscope for staining control (previous to flow cytometry) and biofilm visualization. The FDA and PI signals were obtained by long pass filters: one in the green wavelength range with an excitation bandpass of 470–490 nm and emission at 516 nm, and a second filter in the red wavelength range with an excitation bandpass of 530–550 nm and emission at 591 nm.

Wort sugars and ethanol determination

All sugars and ethanol levels were assessed by high-performance liquid chromatography in a Jasco chromatograph equipped with refraction-index detector (Jasco, Tokyo, Japan) using a Metacarb 67H column (300 × 6.5 mm, Varian) preheated to 80°C by a thermostatted column compartment (Chrompack Instruments AG, Neuheim, Switzerland). The mobile phase (H_2SO_4 0.005 mol L^{-1}) was pumped at a flow rate of 0.3 mL min^{-1} through a Jasco 880 PU pump. Samples were automatically injected (Jasco Intelligent Sampler AS 2057 Plus, USA). Standard calibration curves were obtained using different concentrations of pure sugars (2.5–20 g L^{-1}) and ethanol [1.25–10% v/v; D-glucose (Sigma, UK), D-fructose (Acros Organics, USA), D-maltose monohydrate (Himedia, India), maltotriose (Sigma, USA) and ethanol (Sigma, UK)] prior to wort and green beer samples analyses.

Results and discussion

Biomass growth and biofilm formation

In order to evaluate the consequences of foam formation on biomass losses (biocatalysts and free biomass), the effect of the antifoam agent addition was assessed. The use of the antifoam agent caused a massive accumulation of free biomass (flocculated plus suspended cells) inside the gas-lift reactor (Fig. 2b). This accumulation was the first proof of the negative impact caused by the inhibition of a 'foaming mode of operation', which stops biomass skimming. Additionally, the inhibition of foam formation caused an increase in the size of cell clusters (flocs), which could also contribute to the retention of biomass inside the reactor, as the dilution rate used would not cause its wash-out. Moreover, bearing in mind that the lack of fermentable sugars promotes yeast flocculation *(30–32)*, the lower amount of sugars supplied in this non-foaming experiment (5–11 °P) compared with that fed in the foaming experiment (15 °P) may also have caused higher flocculation, and hence lower biomass losses. Although calcium concentration *(33)* and pH *(34)* may also interfere with flocculation, the possible impact of these two parameters was not evaluated in this work.

The foaming experiment exhibited a completely different behaviour regarding the free biomass profile (Fig. 2a). Owing to the continuous biomass removal through the foam, the free biomass initially decreased (from 10 to 6 g L^{-1}) and then remained almost constant until the end of the continuous culture. Considering that both yeast strains tested were flocculent in batch cultures, the differences observed in biomass accumulation could probably be ascribed to the different fermentation conditions and wort composition, rather than to differences in their flocculation capability *(32)*.

Whether or not an antifoaming agent was used, the maximum cell load achieved in the bioreactor was 0.51 ± 0.086 g of immobilized yeast per g of LCYC. This was consistent with previous work, which had shown that yeast adhesion to LCYC in a gas-lift reactor reached its maximum loading after few days and remained quite stable during the continuous fermentation *(1,25)*. Fig. 3 shows the biofilm formation on LCYC used in this work.

Sugar consumption and ethanol production

During foaming experiments, it was observed that at the same time as glucose and fructose were almost completely consumed, approximately 65% of the maltose and 46% of the maltotriose from the original wort sugars were still present in the young beer (Fig. 2 – a, II). This occurred because the monosaccharides in the wort (glucose and fructose) were preferentially consumed over maltose and maltotriose *(35–39)* owing to direct repression by glucose over gene expression involved in maltose utilization *(40,41)*. The time required for wort fermentation would be greatly reduced if the brewing yeast could ferment maltose at the same time as glucose and fructose *(36–38)*.

Specific saccharide consumption rate (r_s) and volumetric ethanol productivity (r_p) can be useful tools for comparing different reactor systems *(8,42)*. The former considers the difference of sugar concentrations between inlet and outlet of the reactor, thus referring to the amount of sugar consumption per litre of bioreactor volume per hour. The latter considers the difference of ethanol concentrations between the same points, being that at the inlet the ethanol concentration is considered to be null. Additionally, the ratio between the values of the r_s and the inlet saccharide loading rate provides valuable information about the capacity of sugar attenuation of the setup in a given moment. Yet this ratio should be kept within the desired real attenuation, which is normally around 70% for a regular lager primary fermentation *(35,43)*. As dilution rate was constant, during the foaming experiment the saccharide loading rate was 5.48 g L^{-1} h^{-1}, while for the non-foaming experiment it varied from 1.53 (5 °P phase) to 3.25 g L^{-1} h^{-1} (11 °P phase) (Fig. 2).

The average r_s measured in the current work can be found in Table 1. Through the steady phase of foaming experiment a stable average total r_s (considering all fermentable sugars present in wort) of 3.62 ± 0.33 g L^{-1} h^{-1} was measured for several days. This indicates that an average of 66 ± 6% of all fermentable sugars was being used throughout the primary continuous fermentation in the foaming setup. Thus, real attenuation in that

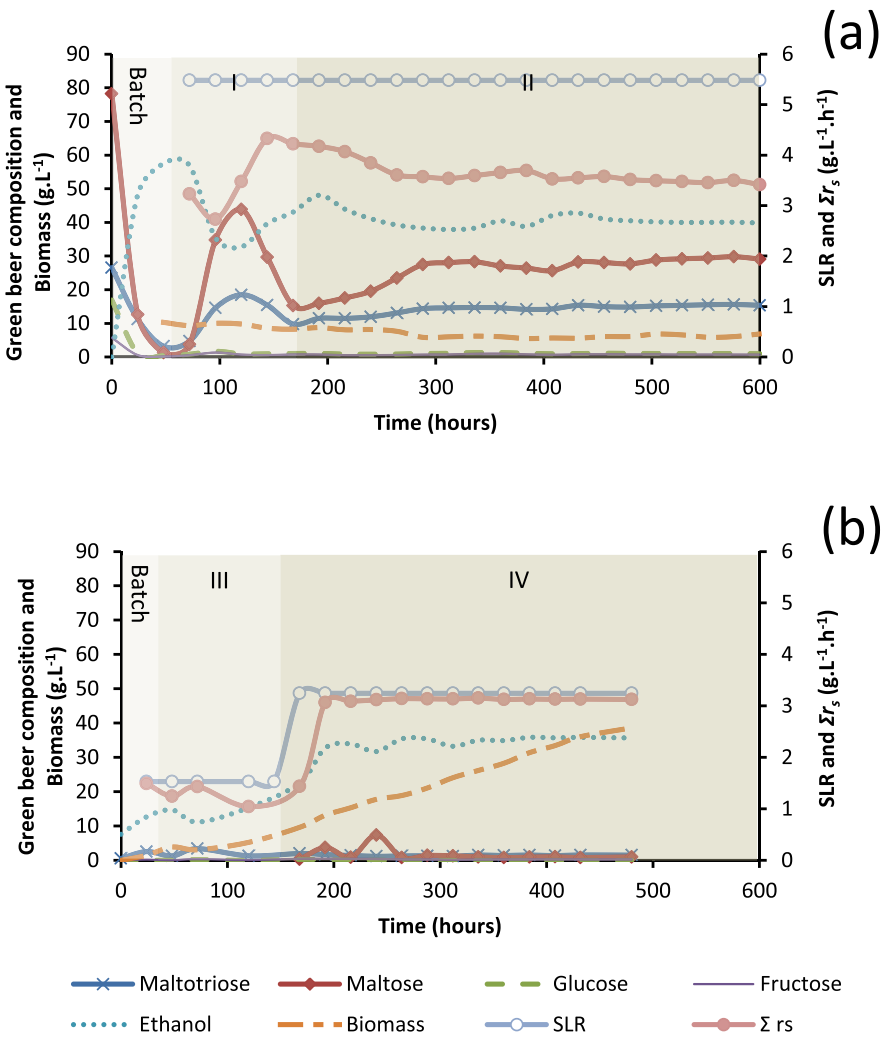

Figure 2. Composition of the green beer and total free biomass concentration in the bioreactor for (a) foaming and (b) non-foaming trials. (a) The initial phase (I) of foaming experiment, marked by a loss of yeast carriers, was followed by a stabilization phase (II) associated with a steady-state consumption of sugars and ethanol production from 15 °P wort. (b) The start-up phase of the non-foaming trial (III) carried out with 5 °P wort was associated with a slight increase in biomass and followed by a high increase in ethanol and biomass (IV) after 11 °P wort supply. (SLR) Saccharide loading rate; (Σr_s) total saccharide consumption rate.

phase oscillated within the desired 70%, but further fine-tuning of the dilution rate would be recommended. Conversely, during the non-foaming experiment, the yeast overpopulation led to sugar exhaustion (96 ± 1%; Fig. 2b). Considering that the dilution rate was kept constant at 0.043 h^{-1}, it was predictable that the supply of sugars would be consumed completely by the growing yeast population. In order to ensure a desirable residual sugar concentration in green beer, increasing free biomass concentration in the continuous reactor should be accompanied by a gradual increase in the dilution rate. In addition to the difficult on-line process control of such a non-equilibrium system, the increasing biomass load would lead to both engineering (high viscosity, mixing and mass transfer limitations) and yeast physiological (accumulation of dead cells, autolysis and ethanol stress) problems. In addition, the use of an antifoaming agent would also certainly negatively affect the foam stability of the final product. The maximum r_s obtained by Smogrovicová and co-workers *(44)*, using yeast entrapment on calcium pectate and calcium alginate in a gas-lift reactor, varied from 5.64 to 6.08 g L^{-1} h^{-1}, respectively, although these authors also reported up to 29 g L^{-1} of residual sugars present in the outflow of the reactor. Such high values of r_s can be ascribed to mono- and disaccharide rich worts, which may have a faster consumption rate than of maltotriose. If cell entrapment technology provides a higher concentration of immobilized biomass inside a reactor, then it can also be restrictive in terms of carrier cost and cell viability maintenance. Tata *et al. (8)* proposed a couple of different reactor setups: a two-stage fluidized bed reactor and another composed of a two-stage reactor containing a silicon carbide cartridge. The average r_s for both reactor systems used by these authors varied from 1.9 to 2 g L^{-1} h^{-1}. Using a similar setup as in the current work, Brányik *et al. (45)* obtained relatively stable r_s values (3–4 g L^{-1} h^{-1}) during almost 2 months of continuous primary beer fermentation. The authors noted that the maximum r_s measured (4.2 g L^{-1} h^{-1}) during the experiment was considerably higher than the average r_s (0.8 g L^{-1} h^{-1}) observed in a batch fermentations with similar wort attenuations.

During the steady-state phase of the foaming experiment, an average of 40 g L^{-1} of ethanol was continuously produced, thus for this phase of the foaming experiment, the average volumetric ethanol productivity was 1.73 g L^{-1} h^{-1}. However for the retention time used in this experiment (23.25 h), there were sugars still present in the outflow current, suggesting that r_p could be further improved for higher values of the

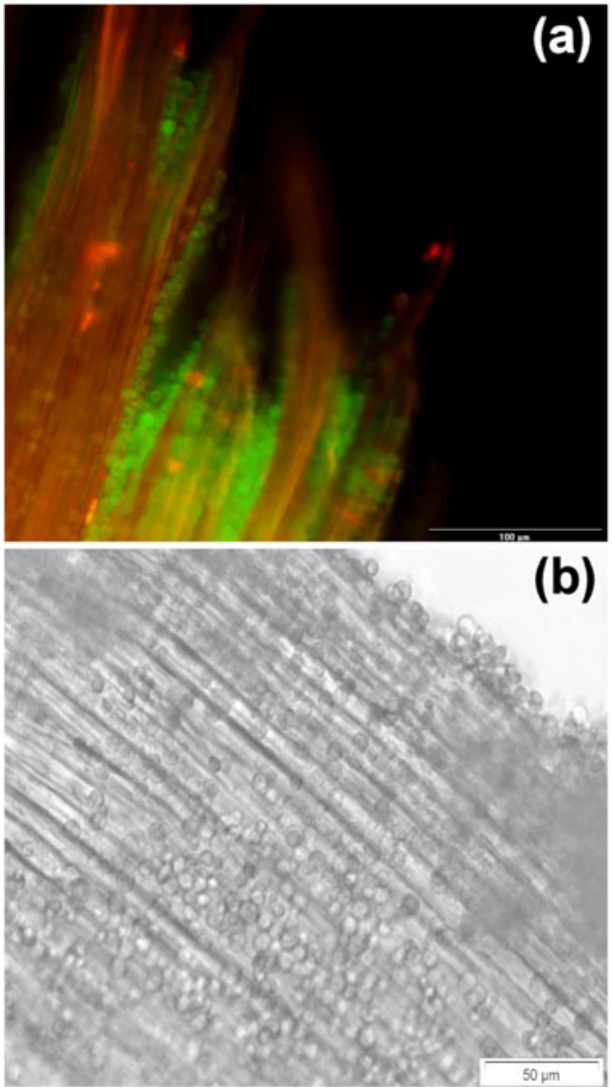

Figure 3. Photomicrographs of yeast biofilm on the lignocellulosic yeast carriers. (a) Double staining with FDA (green cells with esterase activity) and PI (red cells with compromised plasma membrane) of a biocatalyst at the end of foaming experiment. (b) Biofilm visualization using an optical microscope.

Table 1. Average volumetric saccharide consumption (r_s) and average volumetric ethanol productivity (r_p) measured during the steady-state phase of both the foaming and non-foaming experiments

	Foaming	Non-foaming
r_s Maltotriose (g L^{-1} h^{-1})	0.52 ± 0.08	0.43 ± 0.07
r_s Maltose (g L^{-1} h^{-1})	2.2 ± 0.26	1.87 ± 0.21
r_s Glucose (g L^{-1} h^{-1})	0.68 ± 0.01	0.47 ± 0.02
r_s Fructose (g L^{-1} h^{-1})	0.21 ± 0.01	0.18 ± 0.01
Σ r_s (g L^{-1} h^{-1})	3.62 ± 0.33	2.95 ± 0.30
r_p Ethanol (g L^{-1} h^{-1})	1.73 ± 0.12	1.33 ± 0.21

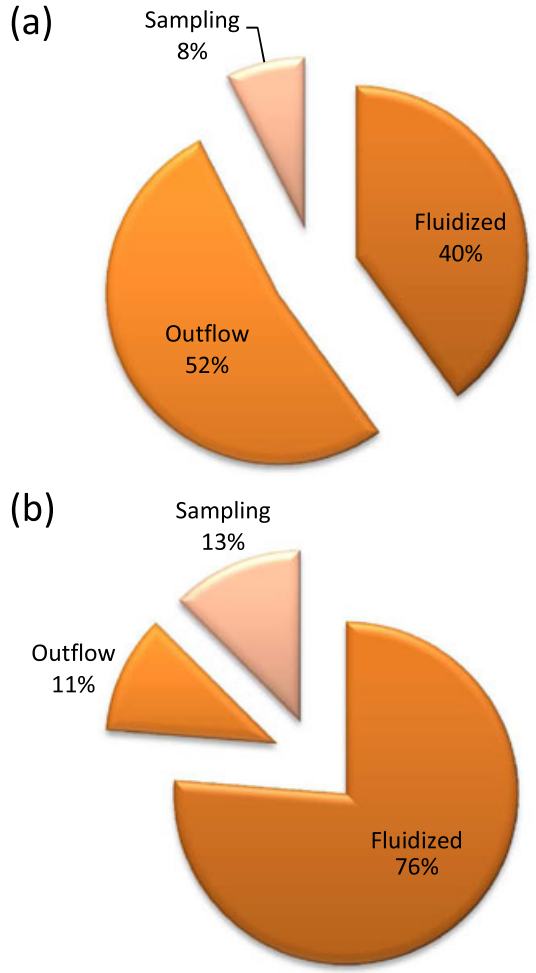

Figure 4. The lignocellulosic yeast carrier distribution and loss during (a) foaming and (b) non-foaming experiments. The fluidized population represents the amount of carriers still suspended in the reactor at the end of the fermentation. Outflow and sampling represents the losses of carriers by these means.

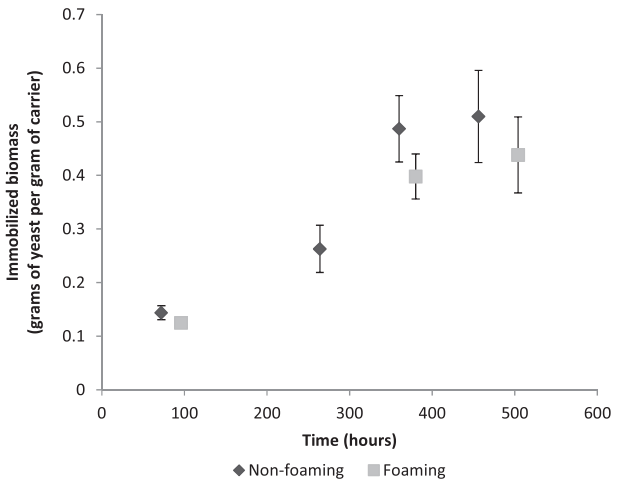

Figure 5. Immobilized biomass concentration measured during experiments.

retention time. The average r_p measured during the non-foaming experiment was lower (1.35 g L^{-1} h^{-1}) than that observed in the course of the foaming experiment. In 2002, Brányik *et al. (2)* used a similar setup for the continuous primary beer fermentation of a 13 ºP wort and obtained an average r_p of 1.6 g L^{-1} h^{-1}. Later in 2004, Brányik et al. *(45)* kept r_p near 2 g L^{-1} h^{-1} for almost 2 months using a 14 ºP wort. Smogrovicová *et al. (44)* used entrapped yeast on calcium alginate and calcium pectate in a gas-lift reactor and obtained r_p values from 1.69 to 2.4 g L^{-1} h^{-1}.

Table 2. Yeast viability assessed by esterase activity and preservation of plasma membrane integrity of different cells sub-populations as determined by double staining with fluorescein diacetate (FDA) and propidium iodide (PI)

Experiment	Cell Sub-population	Stained yeast (%)[a]		
		FDA(+)PI(−)	FDA(−)PI(+)	FDA(−)PI(−)
Foaming	Flocculated	98.7 ± 0.2	1.0 ± 0.09	-
	Immobilized	97.4 ± 0.2	2.0 ± 0.1	-
	Foam	91.0 ± 0.8	8.5 ± 0.3	-
Non-foaming	Flocculated	73.1 ± 0.5	15.4 ± 0.4	11.6 ± 0.6
	Immobilized	62.6 ± 0.7	17.5 ± 0.9	19.9 ± 0.3

[a]Numbers obtained from an average of 20,000 cell counts, performed in triplicate.

LCYC losses and yeast viability

Dead cells will naturally accumulate in the gas-lift reactor if only free suspended cells leave the reactor from the outflow, while the flocculated and immobilized yeast are retained behind the sedimentation barrier of the reactor's outflow *(15,17,18)*. To avoid this situation, regular replacement of LCYC is required, which allows for the formation of new biocatalysts and for the preservation of yeast activity during the wort fermentation inside the gas-lift reactor *(1–3,25,46)*. In the present work however, there was no replacement of biocatalyst by fresh LCYC in the reactor, since the experimentation was aimed at evaluating the losses of the carrier during either foaming or non-foaming fermentations. Regarding these losses, 21 g of LCYC was washed away from the reactor during the foaming experiment, that is, 52.5% from the initial carrier load. Most of these losses were observed during the first week of continuous operation (data not shown). On the other hand, during the non-foaming experiment, only 4.5 g (11.25%) of LCYC was retrieved from the outflow. A detailed distribution of LCYC at the end of both fermentations can be seen in Fig. 4. The massive loss of carrier during the foaming experiment can be in large part explained by the hydrophobic character of the LCYC *(29)* with its tendency to adhere to CO_2 bubbles, and to therefore be dragged out of the reactor.

The concentration of LCYC used was 1% (w/v) and, considering the maximum yeast load onto carriers, the maximum concentration of immobilized biomass was approximately 5.1 g L^{-1} for the non-foaming experiment and 4.38 g L^{-1} for the foaming experiment. However, considering carrier losses, the concentration of immobilized biomass in the non-foaming experiment was, at the end, approximately 4.5 g L^{-1}, while for the foaming experiment it was approximately 2.1 g L^{-1}. The immobilized biomass load measured during both experiments is shown in Fig. 5. Although 4.50 g L^{-1} may sound irrelevant when compared with 47.63 g L^{-1} of the free biomass present at the end of the non-foaming experiment, 2.1 g L^{-1} is about one-third of the total biomass (around 6 g L^{-1}) in the foaming experiment.

Cell viability assessed by esterase activity and preservation of plasma membrane integrity was monitored by a FDA/PI double staining protocol on the last day of fermentation for both the foaming and the non-foaming experiments (Table 2). While foam production resulted in great losses of carrier, it helped to ensure that the free biomass had a high content of viable cells until the late stages of fermentation. At 24 days of continuous fermentation there was still a 98.7% FDA-positive PI-negative (FDA + PI−) stained cell population among the flocculated free biomass.

The immobilized cell population in this experiment also exhibited 97.4% FDA + PI − cells, while the foam's cell sub-population displayed 90.9% FD + PI − cells and 8.5% FD- negative PI-positive staining cells (FDA + PI−). These data suggest that a cell sub-population, with loss of plasma membrane integrity, was being washed out through the reactor's outflow at a considerably higher concentration compared with their presence in the free biomass inside of the reactor (0.98%). This result, which presumably indicates a 'positive discrimination' of the viable cells to stay within the bioreactor, supports the initial hypothesis that biomass removal through foaming could serve to keep the biomass load of a continuously operating fermenter in a viable state for a longer period of time.

For the non-foaming experiment, after 24 days of continuous fermentation, 73.1% of flocculated cells and 62.6% of immobilized cells were FDA + PI−, whereas 15.4 and 17.6% of cells were FDA − PI+, respectively. These data support the concept that, without their periodical removal, accumulation of injured and dead cells occurs in the continuous immobilized bioreactor. However, these numbers may be overestimated owing to possible cell starvation caused by the lack of sugars during biomass growth over the constant dilution rate. Yeast cells, which preserve their plasma membrane integrity, are not permeable to PI *(47)*. Some other cells however, may have lost esterase activity and become metabolically affected or inactive, but still have intact membranes, and thus are not permeable to PI. The percentage of these cells (FDA − PI−) was 11.6 and 19.9% in flocculated and immobilized cells fractions in the non-foaming fermentation, respectively. For comparison, this sub-population was less than 0.1% among cells in the foaming experiment.

The accumulation of yeast cells with compromised plasma membrane integrity must be avoided in a continuous beer fermentation, otherwise the sensorial quality of the final product could be compromised.

Conclusions

The production of green beer with a constant composition can only be achieved by carefully controlling the amount and viability of the biomass inside of a reactor. For this purpose, foam fractionation should be encouraged as a natural solution to control biomass in a gas-lift reactor.

Despite the great losses of LCYC through foam formation during fermentation, this experimental set-up showed steady performance (within desired values) of sugar consumption rate and ethanol productivity. Additionally, it was demonstrated that

the flocculated biomass represents the large majority of the total biomass present inside the reactor. Saccharide consumption and ethanol productivity observed in this work were comparable with other primary continuous beer fermentation setups.

Even though small in number, the PI positive cell sub-population was higher in the foam when compared with the free and immobilized cell sub-populations in the foaming experiment. These data strengthen the hypothesis that foam fractionation could be of help for the constant removal of dead or injured biomass from a gas-lift reactor.

Acknowledgements

Eduardo Pires gratefully acknowledges the Fundação para a Ciência e a Tecnologia (FCT, Portugal) for PhD fellowship support (SFRH/BD/61777/2009) and Cristina Ribeiro from the Centre of Molecular and Environmental Biology/Department of Biology for technical support. This work was also supported by FEDER through POFC – COMPETE and by national funds from FCT through the project PEst-C/BIA/UI4050/2011. The materials supplied by UNICER Bebidas, S.A. (S. Mamede de Infesta, Portugal) are gratefully acknowledged. The financial contributions of the EU FP7 project EcoBioCAP – Ecoefficient Biodegradable Composite Advanced Packaging, grant agreement no. 265669 as well as of the Grant Agency of the Czech Republic (project GAČR P503/12/1424) are also gratefully acknowledged.

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