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CONTINUOUS FERMENTATION OF ALCOHOL-FREE BEER: BIOREACTOR HYDRODYNAMICS AND YEAST PHYSIOLOGY

André Mota, António Vicente, José A. Teixeira
Department of Biological Engineering
Email: amota@deb.uminho.pt

ABSTRACT

Over the last years the development of new beer types such as alcohol–free beer has been increased. This development is motivated by the effort for a greater market share in the brewing industry and by the global trend to a healthier lifestyle without alcohol. Alcohol-free beer can be produced by removal of alcohol after regular beer fermentation or by methods that suppress alcohol formation. Suppression of alcohol formation or its early interruption, so that the alcohol content remains below 0.5 % (vol./vol.), is economically more attractive, since no additional equipment is necessary. Continuous fermentation using immobilized yeast cell systems have been developed successfully allowing the production of regular beer in as little as 2-3 days (Brányik et al. 2004). Similarly to regular lager beers, the production of alcohol-free beer can also take the advantage of continuous immobilized cell systems. In this case, only a short contact between yeast and the wort is required to remove the undesired worty off-flavours (Van Iersel et al. 1995). However, continuous fermentation systems based on immobilized cell technology, have serious limitations as: (1) engineering problems (reactor design, optimization of operating conditions); (2) unbalanced beer flavour (altered cell physiology, ageing) and (3) unrealized cost (carrier price, unstable operation) (Brányik et al. 2005). In order to implement continuous fermentation systems for beer production, further development in reactor design and understanding of immobilized cell physiology, together with application of cheap, alternative carrier materials could provide a new stimulus to both research and application is required (Brányik et al. 2004).

Being so, the main aims of this work are not only to understand and develop an appropriate system (carrier; reactor design, yeast strain) for immobilized yeast in order to produce alcohol-free beer continuously but also to determine and characterize the hydrodynamic behaviour of the reactor. The final objective is to understand the interaction between biofilm formation (immobilization method) and the reactor hydrodynamics in continuous alcohol-free beer fermentation.

At the beginning, the best reactor configuration as well as the best immobilization conditions were selected. Three main goals were addressed: (1) compare immobilization capacity of spent grains with corncobs; (2) chemical modification of both carriers in order to improve their adhesion properties; (3) for each carrier, selection of the best reactor configuration.

As expected, yeast cells were able to adhere either on corncobs (poster presentation) and spent grains. However loading capacity of spent grains revealed to be better in all tested conditions, *i e.* with or without chemical modification of the carrier. The chemical modification was performed with DEC (2-diethylamino-ethylchloride hydrochloride) solution and a slightly improvement on the immobilization was observed. It was also noted that the chemical modification process increases carrier cost and preparation time, making useless this modification.

The two main reactors used for continuous fermentation using immobilized technology for alcohol-free beer production are the packed-bed reactor and the airlift reactor (Brányik et al. 2004; Van Iersel et al. 1995). These two reactors together with both carriers and two different yeast strains were tested in order to decide the best configuration for continuous alcohol-free beer production. In terms of flavor formation, it was shown that the laboratory yeast strain with disruption in the *KGD2* gene performed equally well in batch and continuous packed-bed reactor. However, it was unable to form biofilm around spent grain particles and therefore its use was not possible in the gas-lift reactor. Conversely, the bottom fermenting strain (W96) adhered to the solid supports readily, but the formation of flavor active compounds was insufficient with the exception of immobilization onto

spent grains in airlift reactor. This system arrangement proved that a strain, which seems to be less suitable for alcohol-free beer production, can under appropriate conditions, produce an acceptable final product.
Considering these results, the hydrodynamic characterization of the airlift reactor using spent grains as support for the bottom fermenting yeast strain was performed.

A internal-loop airlift with an enlarge degassing/sedimentation top zone was used. The main objectives of the study were: (1) to understand the fundamentals of the hydrodynamic behaviour of the complex three-phase system, (2) to study the influence of carrier particles on flow behaviour. Initially, the particle influence in a bubble column flow was studied (Mena et al. 2005; Zahradnik et al. 1997) in order to mimic the flow in the internal draft tube of the real airlift without the influence of the downcomer flow. It was demonstrated that spent grains destabilized homogeneous flow at lower gas values than the two-phase system (gas-liquid). Moreover, it seems that above a concentration of spent grains of 12% ($wt_{WET\ BASIS}$/vol.) it was not possible to obtain a homogeneous flow through the bubble column.

Optimization of the airlift design for this particular system was also required. Previous works showed that riser configuration (diameter, length) is one of parameters that most affect the airlift hydrodynamics (Klein, et al. 2003). Being so, studies determining global hydrodynamic characteristics using these particular solids were preformed in order to achieve the best riser configuration for our system. Among the three types of riser configuration studied the riser with small diameter but larger length showed better results in terms of global hydrodynamic characteristics. When compared with other particles (Freitas and Teixeira 1998) and configurations, the absence of a significant radial and axial distribution of spent grains in the reactor was observed together with a reduced solids influence on gas hold-up and smaller mixing times.

Following the design of the airlift system based on the hydrodynamic properties, it is now necessary to understand and described the local hydrodynamic characteristics – an optical fibber system will be used - and how these are related with the biofilm formation.

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