K. Wackerbauer, A. Ludwig, J. Möhle and J. Legrand

# Measures to improve long term stability of main fermentation with immobilised yeast

**Although immobilised main fermentation has clear advantages, insufficient long-term stability of product quality is the main reason that it is still awaiting its breakthrough. Therefore immobilised main fermentations in pilot scale with varying reactor types, carrier materials and aeration rates were applied in order to evaluate measures to improve long-term stability. A comprehensive set of data including beer analyses, taste testing and analyses of yeast vitality during fermentation was collected. It is shown that long term stability is manageable, and a toolset of technological measures is presented to overcome the existing limitations of this technology.**

BC 23 Fermentation/41 Brewers yeast/38 Testing plants

(Descriptors: Long term stability, flavour substances, aroma compounds, immobilised main fermentation, yeast vitality, carrier materials, reactor types.

Deskriptoren: Langzeitstabilität, Aromastoffe, Aromaverbindungen, Immobilisierte Hauptgärung, Hefevitalität, Trägerstoffe, Reaktortypen)

## 1. Introduction

Although main fermentation with immobilised yeast is promising and a lot of efforts have been made to establish this technology it is still in the stage of research. An important reason for this fact is that the product quality is not as uniform as desired over the operation period of a reactor and as it is supposed to be in the ideal case of continous fermentation. Additionally it remains questionable whether the quality of beer produced in an "Immo"-main-fermentation will match the required specifications over a long period of time. Of course main fermentation using immobilised yeast is useful only if the demand for an "optimal and constant product quality during reactor operation" is fulfilled. "Optimal product quality" means that the beer is comparable with existing, batch fermented beers produced for the market. "Constant quality of product" means, that only minor changes of beer quality during the period of reactor operation are acceptable. These demands are fulfilled by a good long term stability of the reactor which is one of the major targets in "Immo"-technology with regard to an excellent product and – last but not least – for economical reasons since the advantages of immobilised yeast systems are only effective if the reactor is able to work for several weeks or even months.

The most important changes in beer quality are related to changes of metabolism and physiological state of the yeast. Table 1 shows developments found in unaerated long term trials applied in our institute during the recent years.

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As far as the experimental design was comparable, similar tendencies were mentioned or reported by several authors [4,9,12]. Here particularly the reduced multiplication of yeast is responsible for the decreased uptake of amino-acids and thus changed formation of fermentation by-products. In consequence, measures have to be taken to promote the growth of yeast to a certain degree to deliver the desired spectrum of fermentation by-products and keep yeast vital. Beside multiplication of yeast, limitations of external and internal mass transfer have an influence on yeast metabolism. Diffusional restrictions due to carrier materials in combination with flow conditions and reactor design appear to play a role in this regard. In our trials different variations were combined in order to evaluate the effect of immobilised yeast fermentation on long term stability.

## 2. Experimental concept

### 2.1 Influence of aeration on long term stability

Suggestions to meet with the inconsistent product quality during reactor operation have been made by several authors. The first and most obvious measure to influence yeast multiplication is to supply yeast with oxygen. This, apart from influencing flavour formation, appears to be useful for yeast vitality in general since unsaturated fatty acids and sterols are exclusively synthesised in the presence of oxygen. These act as essential compounds of the cell membranes necessary for the existence and multiplication of the yeast cell (*Jakobsen* and *Thorne* [8]). *Kronlöf* and *Linko* tested a discontinuous aeration of reactors using a nitrogen-oxygen

**Table 1 Tendencies of development for selected analytical parameters in green beer during aerated reactor operation [6, 22]**

| Analytical parameter | Typical pattern of development in green beer |
|---|---|
| pH-value | Increasing |
| Free amino nitrogen | Increasing, high values after several weeks of operation |
| Vicinal diketones (total amount) | Starting with very high values (> 1 ppm), decreasing considerably by the time |
| Higher alcohols | Decreasing, relatively low level |
| Acetate-esters | Increasing, but relatively low level |

mixture. It was proved that the reactor could be regenerated by such treatment leading to a "corrected" composition of yeast metabolites in beer and improved yeast viability [10]. The bivalence of aeration in continuous systems which can also lead to undesired and unbalanced contents of yeast metabolites and in consequence the necessity of a defined aeration was reviewed by *Norton* and *D'Amore* [14]. Tests dealing with a defined aeration using different gases or gas mixtures in fixed bed fermenters were presented by *Linko* et al. [13], *Virkajärvi* et al. [21] and *Wackerbauer* et al. [23] showing the influence of aeration on the composition of higher alcohols and esters. Finally trials in different semi-industrial plants were carried out with wort aerated before entering the reactor systems [1,11,15,16]. We tried to find out optimal conditions of aeration in order to improve long term stability.

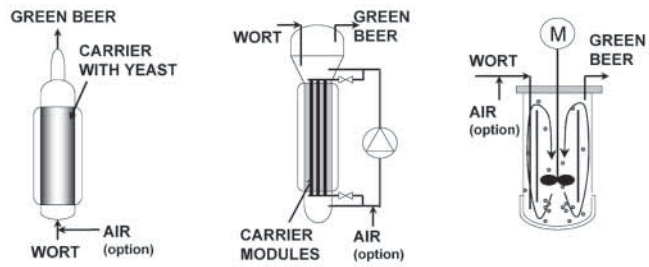


**Figure 1 Principle schemes of the three reactor systems used, from left to right fixed bed-reactor, loop/module reactor and fluidised bed reactor**

#### 2.2 Use of selected carrier materials in fixed bed reactors

The influence of different carrier materials on long term reactor operation with regard to produced beer quality and process behaviour of the carriers was investigated. Restrictions of "internal mass transfer" are strongly related to the carrier [14,17]. In terms of carrier material the following mechanisms of immobilisation can be distinguished: Entrapment within a matrix like Ca-alginate gel, attachment to a solid support surface, adsorption in a carrier cavity and containment behind a barrier (*Cashin* [3]). Although gel-carriers are supposed to be problematic because of the diffusion limitation by the material, *Pilkington* presented gel beads consisting of Kappa-carrageenan applied in a gas-lift reactor [18]. The MPI-reactor developed by the Belgian Meura-Delta-company represents immobilisation behind a barrier since fix silicon-carbide modules were used in this reactor type [2]. Nevertheless most carriers introduced in immobilised yeast systems for brewing are acting with attachment or adsorption as immobilisation mechanism. DEAE-cellulose, porous glass, wood chips and others are commonly used [1, 6, 9 – 13, 15, 16, 21, 22]. Beside the mass transfer, relevant factors for the selection of a carrier material referring to Linko et al. are the following: It should be cheap and easy to handle, the carrier should bind yeast efficiently, should be stable and rigid, should allow smooth plug-flow, must be approved for food use etc. [13]. The differences of the carriers selected for the presented tests regarding the mechanism of immobilisation, their morphology as well as their physical properties were considerable and an influence on reactor operation was probable.

#### 2.3 Comparison of three completely different reactor concepts

By Pilkington et al. and Norton and D'Amore the importance of external and internal mass transfer from the medium to the yeast cells was reviewed comprehensively [14,17]. Mass transfer limitations are an issue with regard to the long term stability of a reactor. Concerning external mass transfer the functional principle of the reactor, for example fixed bed, fluidised bed or agitated is the crucial point since optimised liquid mixing leads to improved mass transfer by minimising the laminar liquid layer surrounding carrier particles. Apart from this clogging and the formation of channels causing a heterogeneous throughput through the system there are obvious disadvantages that may lead to problems in fixed-bed reactors. On the other hand, additional energy costs for pumping or – in case of gas lift fermenters – input of big gas volumes as well as a possible presence of deteriorating shear forces, foaming, and a relatively complex design in general, are disadvantages of fluidised bed reactors. A comparison of fixed and fluidised bed reactors in a brewing application was presented by *Ryder* and *Masschelein* showing a more favourable amino acid uptake and fermentative power of fluidised bed fermentation [20].

### 3 Material and methods

#### 3.1 Reactor sytems and process conditions

In Figure 1 principle schemes of the three reactor systems used are shown. The trials were applied starting with the simplest reactor type, fixed bed reactors. The glass columns were filled with the different carrier materials and sterilised. Yeast coming from a propagator was used for immobilisation. The fermentation conditions with 15 °C and a counter pressure of 1.8 bar (absolute) were kept in all trials that will be mentioned in the following. Wort passed through the reactor bed slowly and flow rate was arranged in a way that almost final attenuation could be reached in order to keep results of beer analysis comparable. The mass flow of wort was approximately 0.5 kg/h for all trials. All reactor systems used were equipped with a connection for an optional aeration. The constant air flow could be adjusted precisely by a needle valve in connection with a flow controller in order to avoid changes of flow rate due to varying reactor pressures.

As another option for variation of reactor system a loop/module-reactor was included in our tests, also known as MPI-reactor manufactured by Belgian Meura-Delta company. This reactor is no new development, but in this system forced flow conditions are realised which contribute to an improvement of diffusional substance transport. Therefore it was included in the specific examinations on long term stability. No granular carrier material or beads are used for immobilisation in this system but porous carrier modules manufactured of silicon-carbide [2]. Yeast is precoated in and on this modules and the substrate is pumped with a relatively high flow rate via a pump loop so that the configuration of a loop reactor is realised. The last reactor system applied was a fluidised bed reactor equipped with a low-shear-force-propeller in combination with an inner tube for effecting the turbulent flow within the reactor. The carrier movement is symbolized by the arrows shown in Figure 1. Even stronger forced flow conditions were adjusted compared to the loop/module reactor investigated before.

#### 3.2 Carrier materials

The following carrier materials were used in the trials presented in this paper:

- ❑ DEAE-cellulose (abbreviated DEAE; Spezyme GDC, Cultor Oy, Finland);

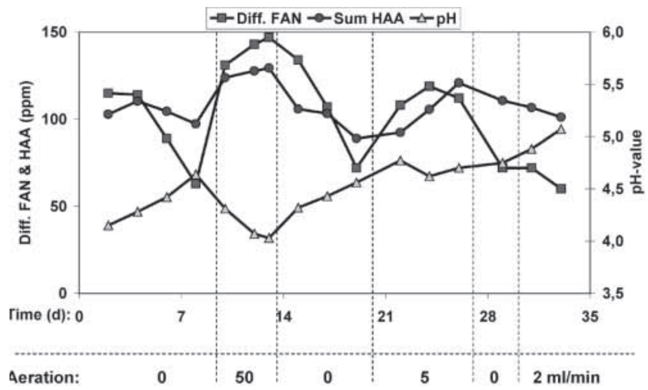


**Fig. 2 Fixed bed reactor (DEAE-cellulose); screening with varying aeration rates**

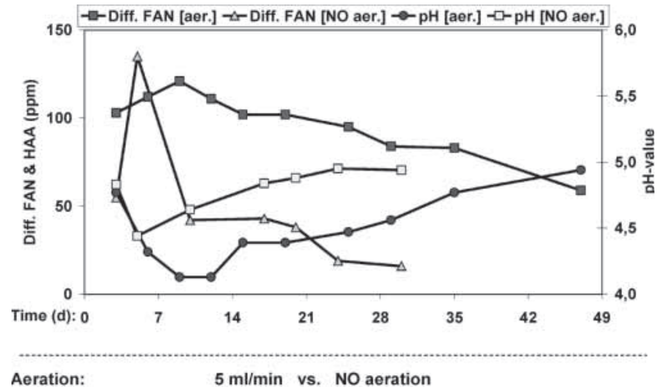


**Fig. 3 Fixed bed reactor (DEAE-cellulose); constant aeration rate vs. no aeration**

- ❑ Sintered glass carrier (abbreviated SGC; SIRAN Sikug 012/05/300, Downing QVF Engineering, formerly Schott Engineering);
- ❑ Silicone carrier; not freely available (abbreviated SIL; TU Braunschweig [5]).

### 3.3 Analysis

Beside conventional beer analysis referring to MEBAK, yeast vitality tests were applied using the determination method of intracellular glycogen described by *Quain* et al. [19]. Intracellular pH-value was applied using the method published by *Imai* et al. [7].

## 4 Results and discussion

### 4.1 Trials with fixed bed reactor

Using the fixed bed reactor with DEAE-cellulose-carrier a screening test with different aeration rates was applied in order to find out a suitable adjustment for subsequent trials. A complete beer analysis was carried out with all samples. Nevertheless data presentation shown in Figure 2 is restricted to some selected values which are suitable to characterise the quality of beer and the situation in the reactor. Below the diagram the adjusted aeration rates in standard millilitres per minute are displayed. Consumption of amino-acids represents the degree of yeast multiplication expressed by the difference of free alpha amino nitrogen (FAN) between wort and green beer in the diagram. With varying aeration rates FAN-consumption was changed. The formation of higher aliphatic alcohols (abbreviated HAA) is displayed, too. The relationship between HAA-formation and FAN-consumption is clearly recognizable since the shape of both graphs is quite similar. The aeration rate found out to be optimal for our test-conditions was 5 ml/min since formation of alpha-acetolactate – which is not displayed here – was moderate. But still an effect on the consumption of amino acids was present at this aeration rate. One interesting fact is that the response of yeast metabolism on changes of aeration is determinable within one day. Therefore aeration could be an interesting additional measure to influence beer flavour in the immobilised system. Furthermore development of pH-value during fermentation shows a certain similarity to FAN-consumptions: Phases of decreased pH-values are always phases of increased FAN-consumption and vice versa. This is due to the intensified formation of organic acids with an increased yeast multiplication.

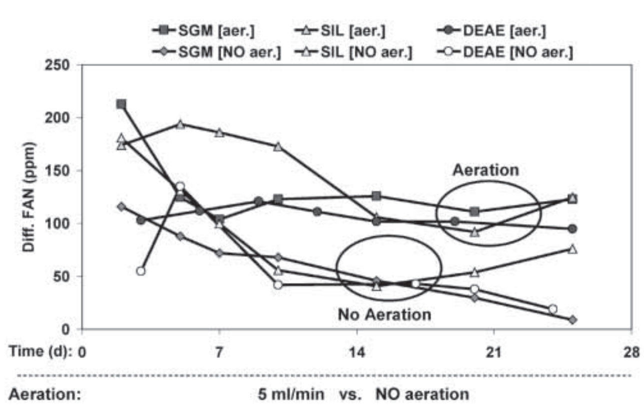


**Fig. 4 Fixed bed reactor (various carriers); constant aeration rate vs. no aeration**

After the screening test a fixed bed trial with a constant aeration rate of 5 ml/min and a reference trial without any aeration were applied. The results are shown in Figure 3. The fast change to low FAN-consumption and high pH-value is obvious for the unaerated trial suggesting a minor long term stability. In comparison the situation is much more stable in the aerated trial with a higher FAN-consumption and lower pH-values even for a considerably longer time of reactor operation showing the improving effect of aeration. Nevertheless a continuous change of beer quality also was present with aeration. After 47 days of operation the beer showed pH-values of almost 5.0 and was unacceptable from the analytical and sensorical point of view. It is assumed that clogging and channel formation in the fixed bed reactor lead to inhomogeneous conditions in the reactor influencing long term stability negatively.

The influence of selected carrier materials with and without aeration was also investigated using fixed bed reactors; the results are presented in Figure 4. For this purpose well known DEAE-cellulose was compared to sintered glass material (SGM) and silicone carrier which originally was used in a waste water treatment application (SIL). The morphology of this carrier was similar to sintered glass material but its consistency is rubber-like and its density is much smaller. Concerning beer quality it can be stated that no significant effect of any carrier was determinable during our trials. If aerated fermentation and unaerated test are compared it can be recognized that the influence of aeration appeared to be much stronger than the effect of carrier material. DEAE-cellulose showed the strongest clogging tendency while sintered glass material was mechanically abridged even in the fixed bed. The silicone carrier was mechanically very stable. Since low density and mechanical stability seemed to be advantageous for use in fluidised bed reactors it was decided to apply the silicone-material in this plant type. Results of this trials will be presented later in this contribution.

### 4.2 Trials with loop/module-reactor

A trial for 13 weeks (Figure 5) was leading to good results concerning beer quality and long term stability with one exception within the first three weeks of reactor operation when an undesired development of increasing pH-value and decreasing FAN-consumptions was found.

Therefore aeration rate was adapted in order to meet the demands. Increase of aeration led to the desired stabilisation of pH respectively consumption of FAN. Between days 50 and 64 after immobilisation the reactor operation was intentionally interrupted and temperature was decreased to 1°C. Beer and yeast remained in the MPI-reactor. As expected, after restart, the first beer leaving the MPI-reactor showed changes in pH-value and FAN-content. Nevertheless within a few days beer analysis was comparable to the situation before reactor operation was paused. The good quality of produced beers was confirmed by sensory analysis. General quality was scored constantly with marks of 3.0 to 3.3 in a 1 to 5 scale during the entire fermentation.

Quite rarely found in literature are observations of yeast vitality in immobilised systems with time-related data as shown in Figure 6. First the content of acid-soluble glycogen in yeast cells was determined during reactor operation. An oscillating behaviour of this storage carbohydrate was found. No steady decrease of glycogen content which could be explained by a restricted nutrient transport into the cell due to impairments of cell membrane was detectable. Neither extremely low glycogen concentrations in general – even after the two week interruption – could be found. During the last phase of operation the analysis of intracellular pH-value introduced by Imai (abbreviated ICP) could be established. For reactor yeast presented here no ICP-value below 6.3 was found. This suggests a favourable situation since much smaller ICP-values of 5.5 were detected in preliminary tests with yeast starving for 24 hours. It can be summed up that glycogen content as well as ICP did not indicate a reduction of yeast vitality. This finding is supported by the results of cell viability not presented here which only showed poor values for the days immediately during restart of reactor after the interruption.

### 4.3 Trials with fluidised bed reactor

During a fermentation test with this reactor a slight decrease of higher aliphatic alcohols and FAN-consumption was found while pH-value increased (Figure 7). In general no critical value was present during the 37 days of fermentation which were observed. Apart from that, it must be considered that aeration rate was not adapted in opposite to the trials with the MPI-reactor presented before. This suggests that the adjusted standard aeration rate of 5 ml/min was not optimal for this configuration. Taste testing of beers produced during this initial trial with the fluidised bed reactor delivered good results. But the constance of this trial was not completely satisfying. Therefore the test programme was extended including especially the adaptation of defined aeration with the aim of a very constant behaviour which is introduced at the end of this text.

The data related to physiological condition of yeast shown in Figure 8 are comparable to the results of loop/module reactor: Oscillating values for glycogen content never showed critically low values. Except for fermentation day number five the intracellular pH-value was quite stable and in a range that suggests no decline of yeast vitality.

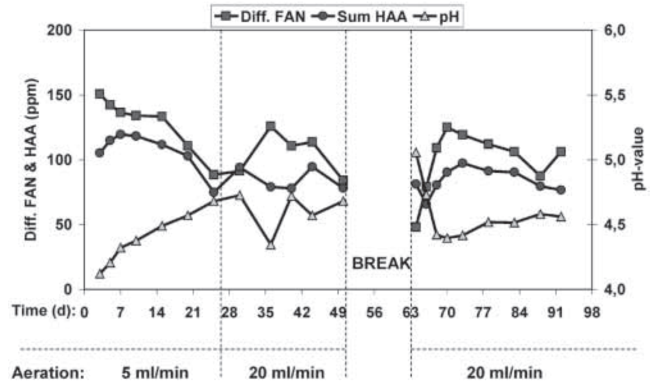


**Fig. 5 Loop/module-reactor; aeration as displayed; selected beer analysis**

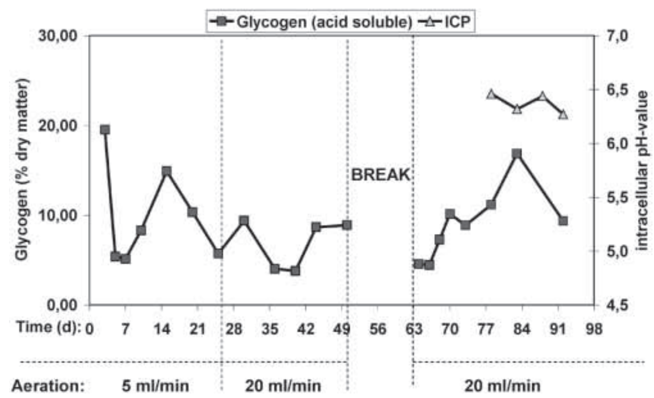


**Fig. 6 Loop/module-reactor; aeration as displayed; yeast vitality**

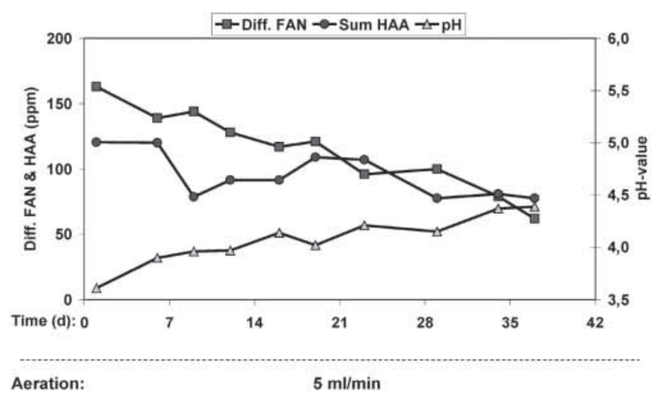


**Fig. 7 Fluidised bed-reactor; aeration as displayed; selected beer analysis**

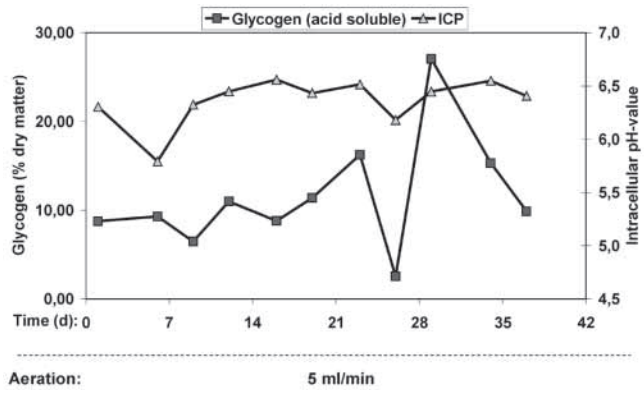


**Fig. 8 Fluidised bed-reactor; aeration as displayed; yeast vitality**

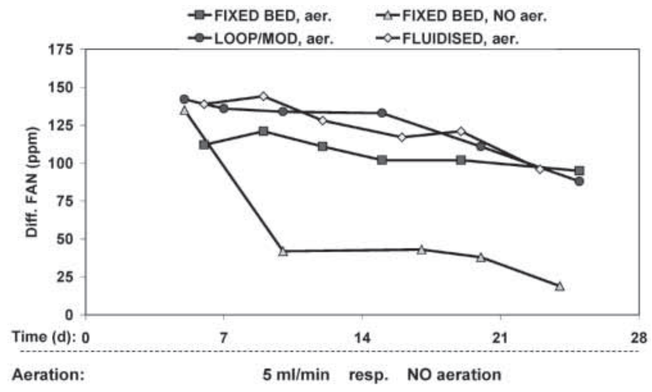


**Fig. 9 Various reactors; aeration as displayed; FAN-consumption**

Finally the results found for the FAN-consumption of different reactor types are summed up in Figure 9. The initial phase of fermentation is not displayed because this phase is always still influenced strongly by propagation of yeast. Data are shown until day 25 when aeration was increased for the trial with the MPI-reactor. As expected, unaerated fixed bed reactor showed the lowest FAN-consumption. Much higher differences between wort and beer were found for the aerated fixed bed reactor. Highest values were found for the loop/module-reactor and fluidised bed reactor which showed similar developments during the observed interval. In terms of pH the fluidised bed reactor showed the lowest values (data not presented).

## 5 Conclusions

In the presented trials applying defined aeration in main fermentation with immobilised yeast it was found that this method is a tool to influence product quality within a short response time. Furthermore defined aeration is a tool to improve long term-stability of immobilised yeast fermentation. There is a reproducible connection between FAN-consumption and pH-value during "Immo-fermentation" which appears to be interesting for a flavour control by aeration. Only a minor influence of three selected carriers on beer quality but considerable differences in process behaviour of the certain materials were found in our trials. This does not mean that the parameter "carrier material" plays no role for the development of beer quality in general. But obviously the influencing factors were minor for this selection of materials. Fixed bed reactors appeared not to be optimal for a main fermentation. Clogging and channel formation impaired overall results with all used carrier materials. For beer quality the best results were found in tests combining forced flow conditions and defined aeration. This is true for the loop/module-reactor as well as for the fluidised bed reactor. In the same tests it was not possible to determine an impairment of yeast vitality. For the future the main remaining problem is an optimised tuning of forced flow and defined aeration which was not solved optimally in the presented tests. This also includes development of procedures to control reactor operation. Our current research is dealing with this controllability issues: For this purpose the fluidised bed reactor is applied, which is equipped with several sensors delivering a survey of fermentation conditions. The reactor is connected with a Siemens Simatic PCS 7-control system which is equipped with a special controller software developed in our research institute. For reactor control the measured pH-value from the reactor is used as process value for the controller. The adjusted setpoint is the desired pH-value of beer. The manipulated value is a calculated aeration rate depending on the measured pH. The higher the pH the more the aeration is increased in order to stimulate yeast metabolism and influence pH as well as related analytical parameters.

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## 6 Zusammenfassung / Résumé

**Wackerbauer, K., Ludwig, A., Möhle, J., und Legrand, J.: Maßnahmen zur Verbesserung der Langzeitstabilität von Hauptgärungen mit immobilisierter Hefe** — Monatsschrift für Brauwissenschaft 56, Nr. 11/12, 210 – 215, 2003

**BC 23 Gärung/41 Brauereihefe/38 Versuchsanlagen**

Obwohl immobilisierte Hauptgärungen klare Vorteile besitzen, ist eine unzureichende Langzeitstabilität der Produktqualität der Hauptgrund, dass sie sich noch nicht durchgesetzt hat. Daher wurden immobilisierte Hauptgärungen im Pilotmaßstab unter Variation von Reaktortyp, des Trägermaterials und der Belüftungsrate durchgeführt, um eine verbesserte Langzeitstabilität zu erreichen. Umfangreiches Datenmaterial wurde gesammelt, das Bieranalysen, Verkostungen und Analysen der Hefevitalität während der Gärung beinhaltet. Es wird gezeigt, dass die Langzeitstabilität beherrschbar ist, und eine Auswahl technologischer Maßnahmen zur Überwindung der bestehenden Unzulänglichkeiten wird präsentiert.

**Wackerbauer, K., Ludwig, A., Möhle, J. et Legrand, J.: Mesures pour l'amélioration de la stabilité à long terme des fermentations principales à l'aide de levures immobilisées** — Monatsschrift für Brauwissenschaft 56, No. 11/12, 210 – 215, 2003

**BC 23 Fermentation/41 Levures de brasserie/38 Installations d'essai**

Bien que la fermentation principale avec levures immobilisées présente des avantages évidents, la cause essentielle n'a pas été trouvée: il s'agit d'une stabilité à long terme insuffisante de la qualité du produit. On a

effectué, pour cette raison, des fermentations principales avec des levures immobilisées à l'échelle pilote en faisant varier le type de réacteur, le matériel de support, le degré d'aération pour atteindre une meilleure stabilité à long terme. On a recueilli un nombre important de résultats comprenant l'analyse de bière, l'analyse sensorielle et l'analyse de la vitalité de la levure pendant la fermentation. On a montré que la stabilité à long terme était maîtrisable et on présente un choix de mesures technologiques pour surmonter les difficultés existantes.

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# Leserdienst

## BC 1 Rohstoffe / BC 3 Chemisch-technische Brauereibetriebskontrolle

### 12 Hopfen / 36 Bier

Xanthohumol, Isoxanthohumol, Hopfen, Polyphenole, Prenylflavonoide, Physiologie

**Forster, A., Gahr, A., Ketterer, M., Beck, B., Massinger, S.: Xanthohumol in Bier – Möglichkeiten und Grenzen einer Anreicherung. Vortrag anlässl. d. 52. Arbeitstagung Bund Österreichischer Braumeister und Brauereitechniker** — Mitteilungen Österr. Getränke Institut 57, Nr. 3, 52 – 62, 2003.

Dem Hopfenpolyphenol Xanthohumol werden positive physiologische Eigenschaften zugesprochen. Die entsprechenden Untersuchungen insbesondere an lebenden Zellen sind zwar noch nicht abgeschlossen, aber trotzdem mag es interessieren, ob und ggf. wie Xanthohumol im Bier angereichert werden kann. Neben konventionellen Pellets oder Hopfentreber nach der $CO_2$-Extraktion gibt es bereits eigens entwickelte Xanthohumol-Extrakte, die gezielt einsetzbar sind. Einer Erhöhung der Gehalte von Xanthohumol und dem Isomeren Isoxanthohumol in handelsüblich filtrierten Bieren sind allerdings enge Grenzen gesetzt. Deshalb unterscheiden sich z. B. untergärige Handelsbiere in den Gehalten von Xanthohumol bzw. Isoxanthohumol nur auf einem niedrigen Niveau von unter 0,1 ppm bzw. unter 1 ppm. Am Ende des gesamten Herstellprozesses von Bier findet sich in die Würze dosiertes Xanthohumol nur mit 10 – 20 % relativ wieder. Soll Xanthohumol wirkungsvoll angereichert werden, empfehlen sich Dosagen mit speziellen Xanthohumol-Extrakten nach der Hauptgärung oder der Hefefiltration, wobei man allerdings mit einer Dauertrübung rechnen muß. Xanthohumol-/isoxanthohumolgehaltvolle Biere sind folglich aus heutiger Sicht nur als Spezialitäten denkbar. Darüber hinaus können Xanthohumol-Extrakte allen Arten von trüben und leicht bitteren Getränken zugefügt werden, die ihrerseits auch die Basis von Biermischgetränken darzustellen vermögen. red#

### 13 Brauwasser

Wasserversorgung, Eisen(II)-Filtration, Eisen(III)-Filtration, Filterbemessung

**Böhler, E.: Planungskriterien zur Enteisenung durch Filtration über inertes Filtermaterial** — GWF Wasser – Abwasser 144, Nr. 9, 589 – 597, 2003.

Die Eisen(II)-filtration über inertes Filtermaterial ist eines der am häufigsten angewandten Verfahren zur Wasseraufbereitung. Die Bemessung kann und soll auf der Grundlage von Versuchen an großtechnischen Anlagen und mit kleintechnischen Filtern vorgenommen werden. Bei Wasserwerken mit einer geringen Kapazität und unproblematischer Rohwasserbeschaffenheit ist der Aufwand für kleintechnische Filterversuche meist unvertretbar hoch. Es wurde deshalb seit den 60er Jahren versucht, Gleichungen für eine freie Bemessung zu entwickeln. Die Entwicklung dieser Bemessungsgleichungen wird erörtert und auf Grundlage einer neuen Auswertung zahlreicher Daten eine erweiterte Bemessungsformel abgeleitet. red#