I. Virkajärvi, K. Lindborg, J. Kronlöf and E. Pajunen

Effects of aeration on flavor compounds in immobilized primary fermentation

Continuous immobilized primary fermentation has been studied since the last century, but has not yet fulfilled expecta-tions. There are two main biological difficulties for continuous primary fermentation: achieving and maintaining the desired flavor and preventing contaminations. One traditional way of controlling the flavor of beer is by aeration of wort prior to pitching. We explored the effects of aeration in immobilized primary fermentation. The fermentation system used consisted of two packed bed reactors and a buffer tank between the reactors. An industrial wort and an industrial brewer's yeast strain were used. The wort was aerated by mixing air into the wort stream just before the inlet of the first reactor (pre-column). The air was diluted with carbon dioxide and both were filtered twice through a PTFE 0,2 µm membrane (domnick hunter Ltd., Durham, UK). The feed rates of synthetic air and carbon dioxide were changed independently according to a Box-Hunter experimental design while keeping the feed rate of the wort constant. The results were analyzed using a computer program for experimental design Modde 3.0, (Umetri AB, Uppsala, Sweden) and mathematical models for the concentrations of higher alcohols and two acetate esters in the out-flow from the pre-column were created. It was found that both low air feed and high air feed with low carbon dioxide feed stimulated the 3-methyl butyl acetate production in the pre-column. The main column seemed to even out the changes in the aroma compounds analyzed, so that their final levels were relatively insensitive to air supply in the range studied.

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(Descriptors: Flavor compounds, immobilized primary fermentation, mathematical model.

Descriptoren: Geschmacksstoffe, Aromakomponenten, immobilisierte Hauptgärung, mathemtisches Modell).

1 Introduction

Continuous main fermentation of beer was described already in 1892 (Delbrück 1892), but despite many trials in the 1950's and 1960's almost all processes were abandoned by the end the 1960's. The interest for continuous fermentation of beer revived in the early 1970's after the paper by Narziss and Hellich (1971) where immobilized primary fermentation was described. Immobiliza-tion seemed to solve some of the problems associated with continuous primary fermentation such as limited number of suitable yeast strains. Continuous operation offers economical advantages, but in the brewing process some obstacles have to be cleared. Exact flavor matching with the existing product seems to be of major importance.

Continuous fermentation with immobilized yeast has been plagued with too low ester levels (Ryder and Masschelein, 1985, Cop et al., 1989). The fact that wort aeration affects the flavor formation in batch fermentation especially for esters is widely documented in the literature (e.g. Nordstedt, et al. 1975, Knatchbull and Slaughter, 1987). Kuriyama and Kobayashi (1993) have studied the effect of aeration on ester formation in a continuous fermentation.

Autoren: Ilkka Virkajärvi, Katri Lindborg, VTT Biotechnology and Food Research, P.O. Box 1501, FIN-02044 VTT, Finland, Jukka Kronlöf, Oy Hartwall Ab, P.O. Box 44, FIN-15101 Lahti and Esko Pajunen, Oy Sinebrychoff Ab, P.O. Box 87, FIN-04201 Kerava, Finland

2 Materials and methods

2.1 Reactor system

The equipment is presented in Figure 1. The packed reactors were of glass. The carrier was porous glass beads from Schott Engineering GmbH, Mainz, Germany (Siran SIKUG 023/A). The diameter of the beads was 2 to 3 mm and porosity 50 to 65%. The prereactor was slightly conical in shape and had 3,5 dm^3 carrier in a total volume of 5 dm^3. The main reactor was cylindrical and had 20 dm^3 of carrier in a total volume of 25 dm^3. Both reactors were operated with upward flow. The prereactor feed was aerated with a mixture of carbon dioxide and synthetic air (AGA Oy Espoo, Finland).

Pumps were ProMinent Gamma/4-W positive displacement membrane pumps (Dosiertechnic GmbH, Heidelberg, Germany). Tubing was silicone (inner diameter 5 mm, wall thickness 2 mm).

The system also had a metal wort tank (85 dm^3) and a buffer tank (glass, 20 dm^3) and a receiving vessel (metal keg, 50 dm^3). The buffer tank was needed to remove gaseous CO_2 from the liquid entering the main column. Concurrently it removed most of the yeast from the liquid.

2.2 Starting of the reactors

The reactors were assembled and autoclaved (121 °C, 20 min). The yeast suspension from rotary shaker cultivations in 3 dm^3 conical flasks was pumped upward through one reactor at a time with the ProMinent pump (about 3 dm^3/h). The prereactor was pitched with 6 dm^3 (2 flasks) of suspension and the main reactor with 18 dm^3 (6 flasks) of suspension. After this the reactors were fed with wort at 200 ml/h and aerated with sterile filtered pressurized air (50 ml/min into the prereactor and 200 ml/min into the main reactor) for 16 hours. This yeast propagation stage was performed for the prereactor at 20 °C and for the main

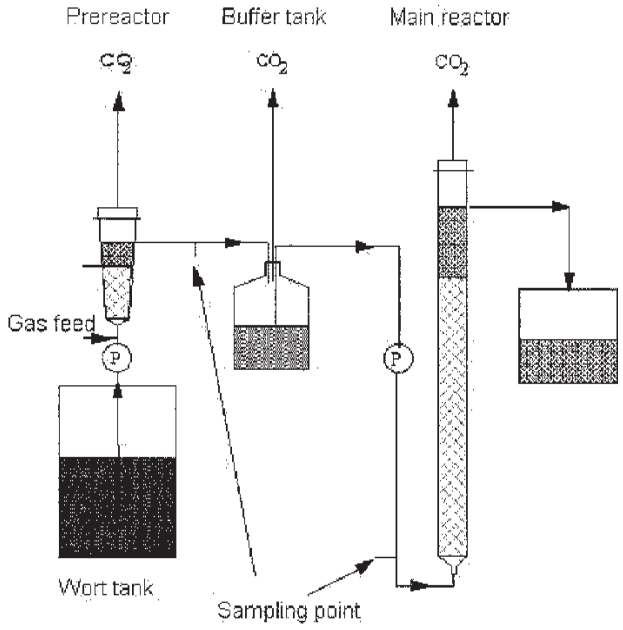


Figure 1 The immobilized yeast reactor system used

reactor at 10 °C for operational reasons. Then the prereactor was transferred to 10 °C and connected to the system. The wort was diluted to 11°P and autoclaved (121 °C, 20 min). The feed rate was 500 ml/h. Into the feed stream of the prereactor 167 ml/min of CO_2 and 4.4 ml/min air (both from AGA Ltd., Espoo, Finland) were fed through a sterile filter (domnick hunter Ltd., Durham, UK, PTFE 0.2 micron).

2.3 Operation of the reactors

Industrial wort was diluted to 11°P with tap water and then autoclaved at weekly intervals. For the duration of autoclaving and subsequent cooling (about eight hours) the feed to the columns was stopped. During this time the pumping from the buffer tank into the main column was at lower rate. After connecting the feed tank to the system the feed pump was started again. This process interruption had no adverse effects on the system (Kronlöf, un-published results).

Every two weeks accumulated yeast was removed from the buffer tank. Both pumps were stopped and the buffer tank was removed and emptied. It was autoclaved before reconnecting to the system. Then the feed pump was started. When the volume of fermenting wort in the buffer tank was restored the second pump was started. This took about 10 hours.

The system was run in this way for 137 days continuously before the present experimental series. This long period stabilized the system and ensured that it could be kept aseptic. Each experiment started with setting new CO_2 and air feed rates without changing the wort feed rate. Each experiment was run for two weeks and the aroma compounds were analyzed once a week. The averages of these measurements were used in statistical analysis.

2.4 The experimental design

To maximize the amount of information from a given number of experiments, an experimental design (Box and Hunter, 1957) was used. The design and resulting mathematical models for aroma compound formation were created using a commercial program (Modde 3.0, Umetri AB, Uppsala, Sweden). A central composite design (Box and Hunter, 1957) was chosen. The method includes the use of coded values of the parameters. One problem with using a central composite design is the rapidly growing number of experiments when the number of parameters investigated is increased. With two parameters the number of experiments is at least 9 (one central, 4 corner and 4 star points). At the center point measurements are repeated in order to achieve either uniform precision or orthogonality. Only one experiment was run at the central point, but aroma compounds were analyzed 5 times during the experiment at the central point. This is a small violation of the normal procedure to randomize the experiments but as each experiment lasted two weeks the total time needed would other-wise have been very long.

2.5 Yeast and wort

An industrial yeast strain (VTT-A85072) from VTT Technical Research Centre, Espoo, Finland, and an industrial wort of 13°P with 30% unmalted cereal adjunct were used.

2.6 Analytical methods

2.6.1 Aroma compounds

The aroma compounds (ethyl acetate, 3-methyl butanol, 2-methyl butanol, propanol, ethyl caproate, 2-methyl propanol, acetalde-hyde, 3-methylbutyl acetate) were analyzed with a Perkin-Elmer Autosystem XL gas chromatograph, a Perkin-Elmer head space autosampler (HS40) and FID detector (Perkin-Elmer Corp., Norwalk, USA). A sample of fermenting beer was centrifuged 10 min at 3770 g and then filtered through a 0,2 µm AcroCap™ filter (GelmanSciences, USA). A 5 ml of the sample was incubated 90 min at 55 °C in a 20 ml glass vial prior to sampling the head space. n-Butanol was used as an internal standard. The injector and detector temperatures were 225 °C and 250 °C, respectively. Helium was used as carrier gas (1 ml/min) and nitrogen as make-up gas (40 ml/min). The column was PE-5, 1 µm film, 50 m (Perkin-Elmer Corp., Norwalk, USA). The temperature program used was: start at 40 °C with 12 °C/min programmed increase to 100 °C with 3.5 minutes hold at 100 °C. Then a second programmed increase 12 °C/min to 150 °C followed by a 5.0 min hold at 150 °C was carried out.

2.6.2 Total diacetyl

A sample of fermenting beer was centrifuged 10 min at 3770 g and then filtered through a 0,2 µm AcroCap™ filter (GelmanSciences, USA). A sample (5 ml) was incubated for 90 min at 60 °C encapsulated in a 20 ml glass vial to convert α-acetolactate to diacetyl. 2,3-Hexanedione was used as internal standard. The analyses were performed with a Perkin-Elmer Autosystem XL gas chromatograph, a Perkin-Elmer head space autosampler (HS40) and an EC-detector (Perkin-Elmer Corp., Norwalk, USA). The injector and detector temperatures were 90 °C and 120 °C, respectively. Helium was used as carrier gas (1 ml/min) and nitrogen as make-up gas (30 ml/min). The column was PE-5, 1 µm film, 50 m (Perkin-Elmer Corp., Norwalk, USA). The temperature

program used was: 35 °C for 2 min, then a programmed increase 6 °C/min up to 100 °C followed by a two minute hold at 100 °C.

2.6.3 Microbiological analysis

Microbiological samples were taken once a week from the wort, the outlets of the pre-column, the buffer vessel and the main column. The possible presence of wild yeasts were monitored with Yeast Medium (Difco 0712) + 0.6 g/l $CuSO_4 5H_2O$ for 4 days at 24 °C (EBC Analytica Microbiologica 4.2.5.1). Aerobic bacteria were cultivated on saccharose agar (40 g saccharose, 10 g maltose, 10 g peptone, 5 g yeast extract, 5 g NaCl, 1 025 g $MgSO_4 7H_2O$, 0.56 g $MnSO_4 H_2O$, 0.1 ml Tween 80, 20 mg bromocresol green, 3 g $CaCO_3$ and 20 g agar in 1 l distilled water) containing also cycloheximide (10 mg/l) for 3 days at 28 °C. Yeast viability in the outlet was measured weekly by methylene blue staining (EBC Analytica Microbiologica 3.2.1.1).

2.6.4 Apparent degree of attenuation

The apparent degree of attenuation was determined by specific gravity measurements (EBC Analytica 9.4.1.3.).

3 Results

The average values for the measured aroma compounds from the prereactor are listed in Table 1. The average values during the first 137 days of fermentation are included in Table 1. No significant microbial contaminations were detected and the apparent degree of attenuation remained stable and close to the attenuation limit of the wort during the experiment.

3.1 Response surfaces

The behavior of the higher (fusel) alcohols in the prereactor was modelled with the Modde 3.0 program. The concentrations of higher alcohols were summed and this number was used in modeling. After removing one outlier (experiment number 14) a second order fit achieved $R^2 = 0.92$ and $Q^2 = 0.55$ (Fig 2). R^2 represents the accuracy of the fit (R^2 less than 0.8 indicates poor modelling) and Q^2 is the predictability ($Q^2 > 0.5$ indicates good modelling).

After examining the confidence intervals for the second order coefficients for terms air*air, CO_2*CO_2 and air*CO_2 these terms were deleted from the model as insignificant thus reducing the model to a linear one. This simplification of the model (Fig 3) reduced the accuracy of the fit to 0.90 (from 0.92) but increased the predictability to 0.82. (from 0.55) So the linear model was as good as the second order one. Either of these two models can be used, though the second order model may prove better if one extrapolates outside the experimental range.

Both models show that with low CO_2 feed rate and high air feed rate the maximum amount of higher alcohols is formed in the prereactor. This is not surprising because high aeration in a batch

Table 1 Parameter values and aroma compounds leaving the prereactor

Exp.	Parameter			Variable					
#	ml/min			mg/litre					
	CO_2	air	IAA	EthAc	ProOH	2-Me ProOH	3-Me BuOH	2-Me BuOH	AcAld
average 0 – 137 days	167	4.4	0.6	12.1	6.1	4.7	17.7	7.1	13.6
1	25.6	2.0	0.6	10.5	7.0	5.8	23.3	8.4	11.2
2	149.4	2.0	0.8	16.1	7.3	6.0	24.1	10.1	11.2
3	25.6	5.0	0.8	12.7	12.5	9.0	31.7	7.0	9.5
4	149.4	5.0	0.7	12.9	9.1	7.9	29.7	10.9	9.3
5	0	3.5	0.7	14.3	11.0	12.7	41.7	16.1	9.7
6	175	3.5	1.2	14.6	8.4	7.3	29.4	11.2	10.6
7	87.5	1.5	1.0	14.1	7.5	7.2	26.2	10.7	10.6
8	87.5	5.5	0.8	12.7	12.3	9.1	34.8	12.9	15.0
9	149.4	5.0	0.7	12.9	9.1	7.9	29.7	10.9	9.3
10	87.5	3.5	0.8	11.6	6.7	6.4	26.6	9.3	11.7
11	87.5	3.5	0.6	9.2	7.7	8.4	30.3	11.2	15.8
12	87.5	3.5	0.6	9.6	11.7	10.3	35.5	12.9	10.
13	87.5	3.5	0.5	7.7	10.8	8.8	31.6	11.2	9.2
14	87.5	3.5	0.6	10.6	15.4	14.2	46.0	17.1	8.6

IAA = 3-methylbutyl acetate, EthAc = ethylacetate, ProOH = propanol, 2-MeProOH = 2-methyl propanol, 3-MeBuOH = 3-methyl butanol, 2-MeBuOH = 2-methyl butanol, AcAld = acetaldehyde

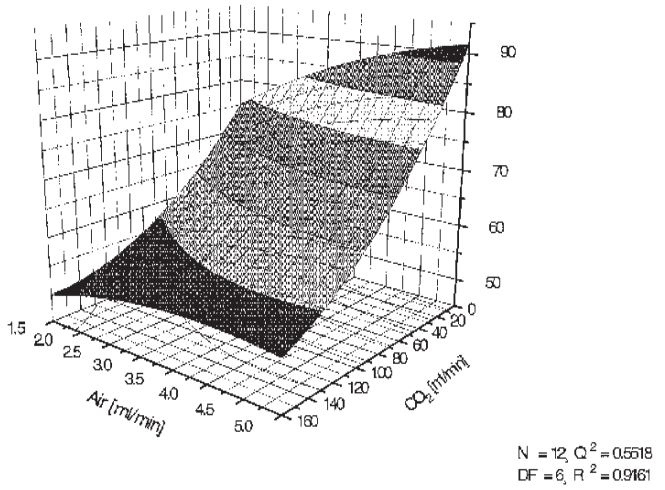


Figure 2 The response surface of the second order model for higher alcohols leaving the prereactor

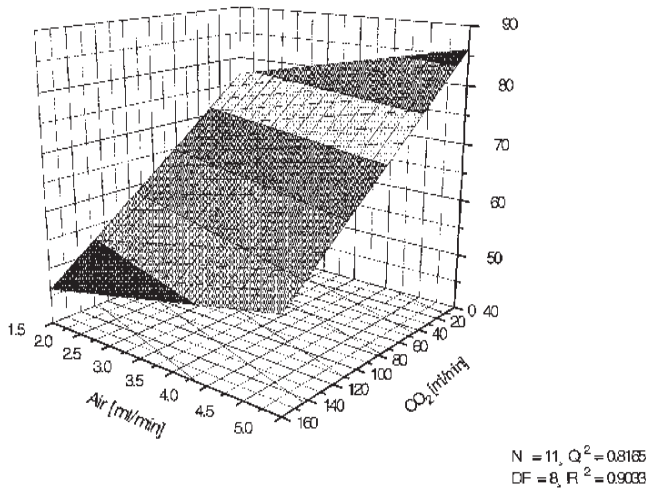
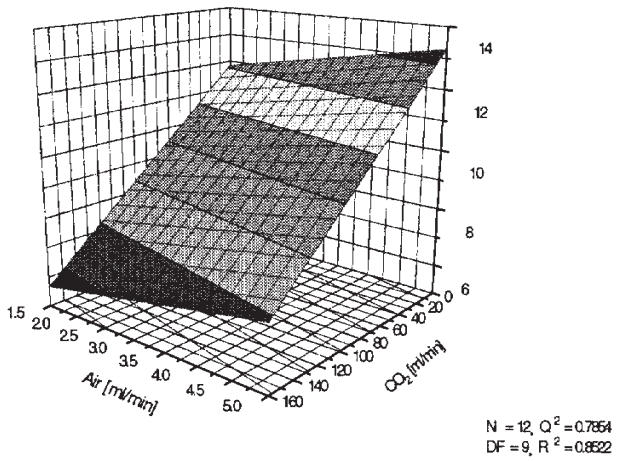
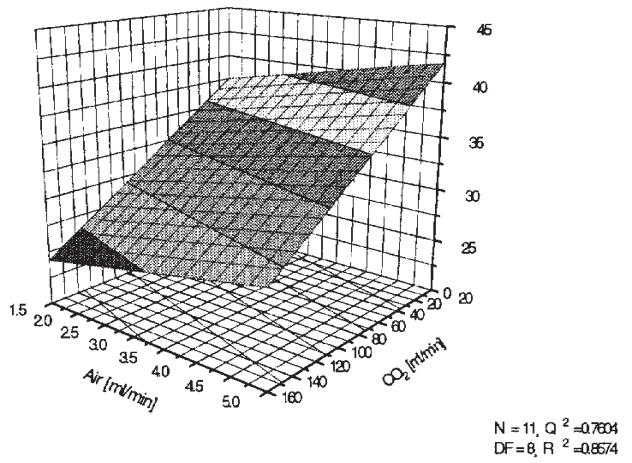


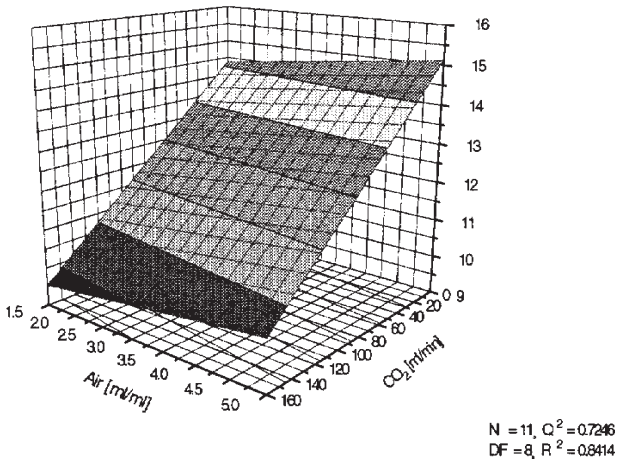
Figure 3 The response surface of the linear model for higher alcohols leaving the prereactor



2-MeProOH



3-MeBuOH



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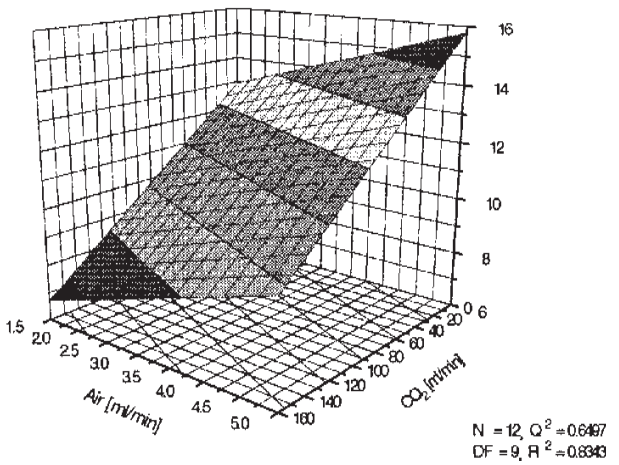


ProOH

Figure 4 The response surfaces of individual higher alcohols leaving the prereactor

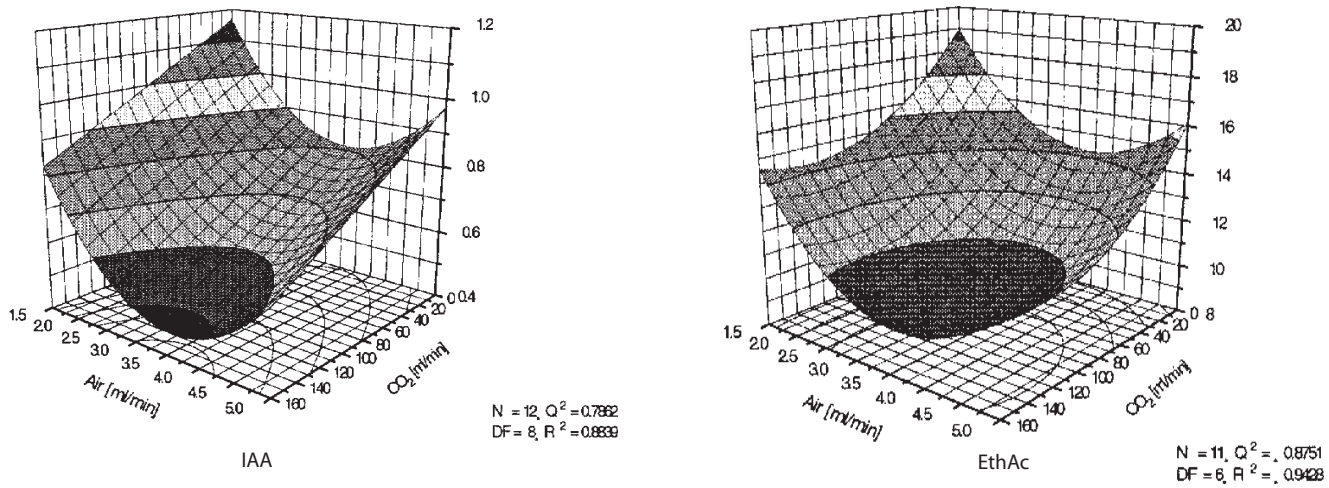


Figure 5 The response surfaces of individual acetate esters leaving the prereactor

fermentation has a similar effect. Kahler et al. (1965) also noticed that higher aeration led to raised levels of higher alcohols and acetaldehyde in a continuous fermentation. It is to be noticed that the high value for the sum of higher alcohols is almost double the low value in the studied range.

Individual higher alcohols are modeled in Fig 4. Propanol proved to be most difficult to model. The R^2 value for propanol was 0.83 and Q^2 value was 0.65.

For esters, the second term coefficients were the significant and determining ones, and so a linear model was not applicable. The respond surfaces for acetate esters are presented in Fig 5. The immobilized system described has had a slightly lower concentration of 3-methylbutyl acetate than corresponding batch fermented beer. Form the second order model for 3-methylbutyl acetate (Fig 5) it can be seen that with low air feed combined with low CO_2 feed the highest concentration of 3-methylbutyl acetate is achieved. At this point the concentration of higher alcohols is high, which may be advantageous. The other maximum (high air, low CO_2) coincides with the highest concentration of higher alcohols.

For ethyl acetate the maximum is found with 1.5 ml/min air, 0 ml/min CO_2. Almost as high value is modeled at 5 ml/min air and 0 ml/min CO_2.

The formation of esters in the brewing process is extensively studied. Nordström et al. (1975) found that the initial amount of oxygen in the wort did not affect the amount of esters formed in a batch process. On the other hand, aeration during the fermentation reduced the ester formation.

Acetaldehyde had very poor accuracy of the fit and predictability in both second order and linear models (Fig 6).

The levels of aroma compounds in the outflow from the second reactor were modelled also, but the same models as for the prereactor did not apply. This is not surprising because the physiological state of the yeast in the second reactor is not expected to be so dependent on the aeration rate. Some yeast will travel with the fermenting wort from the prereactor through the buffer tank into the second reactor thus affecting the state of the yeast in the second reactor. The concentration of aroma compounds entering the second reactor should affect the out-flowing green beer, but it seemed that an oxygen leak through the silicone tubing immediately prior the second reactor affected the behavior of the second reactor. The response surfaces of the sum of higher alcohols and sum of acetate esters are shown in Fig 7.

Maximum concentration of higher alcohols in the young beer (outflow from the second reactor) was achieved at 4.5 ml/min air with 70 ml/min CO_2. This resembles the behavior of the higher alcohols in the prereactor, but the maximum concentration was shifted to lower gas feeds. The acetate esters had their maximum concentration at low air and high CO_2 feed. This is somewhat in contrast with the model for the prereactor, but the difference in the concentration of acetate esters along the low air feed (1.5 ml/min) line is small. So, the second highest value of the model for the second reactor coincides the maximum of the model for the prereactor.

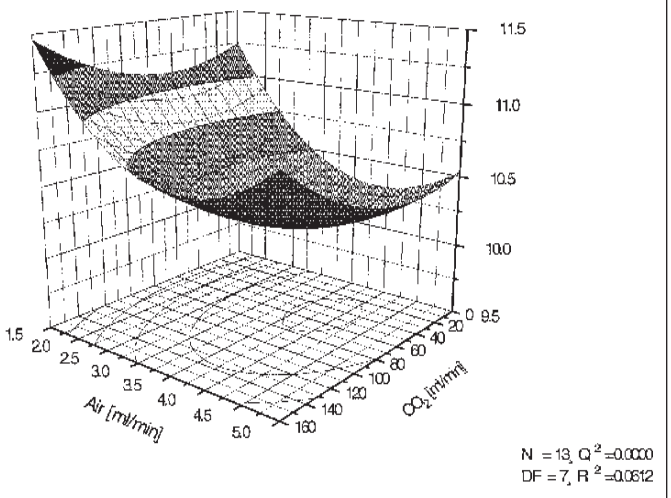
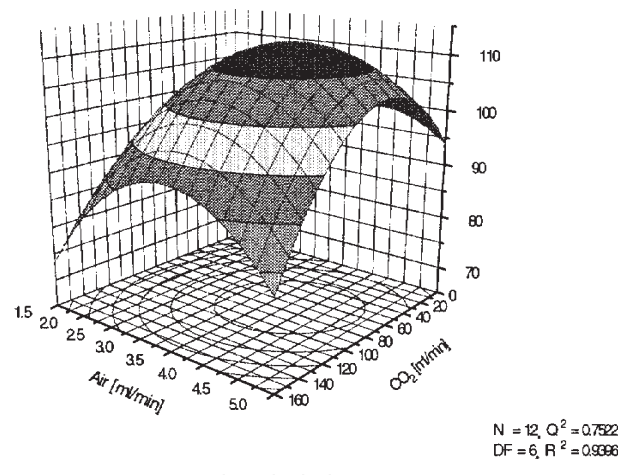
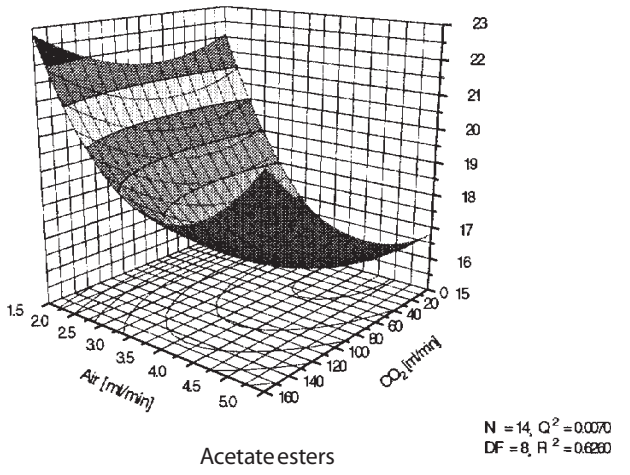


Figure 6 The response surface of the second order model for acetaldehyde leaving the prereactor



Higher alcohols



Acetate esters

Figure 7 The response surfaces of the second order models for the sum of higher alcohols and for the sum of acetate esters in the outflow from the second reactor

4 Discussion

These results show that the formation of higher alcohols and esters can be controlled to some extent by varying the gas feeds into the prereactor. The formation of higher alcohols followed the pattern in a batch fermentation: higher amounts of air in the feed increased the formation of higher alcohols.

Propanol had the lowest accuracy of the fit ($R^{2)}$) and the lowest predictability (Q^2) of the higher alcohols modeled. This is of interest, because the amount of propanol produced with the immobilized yeast reactor system used has been higher than in the corresponding batch fermented beer (Virkajärvi & Kronlöf, 1997). The formation of propanol is different from that of the other higher alcohols because there is no naturally occurring corresponding amino acid, so only the anabolic route from carbohydrates is possible. The excessive formation of propanol, though still well below taste threshold, may be a reflection of lower free amino acid consumption (data not shown) in the immobilized fermentation process. The lower consumption of FAN might increase the anabolic flux. Cop et al. (1989) noticed that with yeast immobilized in porous glass beads the formation of propanol increased with increasing superficial velocity in a fluidized bed reactor. The utilization of FAN was also a linear function of the superficial velocity, but on the other hand they found very small differences in the uptake of individual amino acids as a function of the superficial velocity (Cop et al. 1989). The possibility of controlling flavor formation through FAN-concentration is worth investigating.

Formation of esters is reduced by wort aeration in traditional batch fermentation as it is supposed to reduce the availability of acetyl-CoA for ester synthesis (Anderson & Kirsop, 1974). Aeration has been shown to reduce the enzyme synthesis of alcohol acetyltransferases which are responsible for these esters (Yoshimoto et al. 1998).

A similar effect was seen also in the immobilized fermentation (high ester concentration with low air feed), but high ester concentrations are modelled also at a high air feed when the CO_2 feed was low. Masschelein (1986) found that in an aerated continuous fermentation the level of 3-methylbutyl acetate was 77 times lower that in an anaerobic continuous fermentation (fluidized bed, alginate entrapment). Also, the anaerobic continuous fermentation resembled the discontinuous (batch) anaerobic fermentation. The conclusion was that the availability of oxygen is the critical factor for ester synthesis – instead of fermentation mode: continuous or discontinuous (batch).

Acetaldehyde proved too hard to model reliably: R^2 of 0.06 and Q^2 of 0.00. The reasons for this are unclear, but it may reflect the observed sensitivity of acetaldehyde towards oxygen (Kronlöf and Linko, 1992). Kuriyama and Kobayashi (1993) found that acetaldehyde increased with increasing aeration in continuous fermentation.

The effects of gas feeds on the measured aroma compounds are probably complicated by the problem of oxygen mass transfer from the gas phase into the liquid phase in a packed bed reactor. Possibly also the internal diffusion limitations within the glass beads have an effect. The carrier type has an effect, too (Kronlöf, 1994).

The air feed is necessary to ensure the long term viability of yeast (Kahler et al., 1965), but at least in a relatively small experimental system the air feed must be diluted with N_2 or CO_2 to avoid overaeration which would lead to excessive formation of acetaldehyde (Kronlöf & Linko, 1992). N_2 was originally used, but it was later replaced with CO_2 In a brewery the logical and economical choice may be CO_2 and it does not have adverse effects on fermentation. The effects of CO_2 on growth or metabolism of yeast seem to be negligible in the conditions used (for review see e.g. Jones and Greenfield, 1982).

The response surfaces for the outflow of the second reactor are different from those for the prereactor. The changes in the gas flows affect less the immobilized yeast in the second reactor as all the oxygen is removed from the wort before the second reactor. There may have been some oxygen transfer through the silicone tubing connecting the buffer tank and the second reactor. The effect of the latter is seen in the increase in the acetaldehyde concentration and lowering in the 3-methylbutyl acetate concentration between the buffer tank and the first sampling port (located in the lower part) of the second reactor (data not shown). The unsaturated fatty acid content of yeast

was also somewhat higher that expected in the main reactor after 10 months of fermentation (Lindborg, 1997).

Achieving the desired, balanced flavor of beer produced by an immobilized yeast reactor system is at least as challenging as in a batch process, but a careful control of the air feed into the system makes it possible. Optimization of aeration of fermenting wort is needed. An additional control feature may be in the free amino nitrogen of the wort. Careful reading is needed when comparing results in the abundant literature as the methods of immobilization and reactor designs vary and they are not without influence on the formation of aroma compounds.

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5 Zusammenfassung

Virkajärvi, I., Lindborg, K., Kronlöf, J., und Pajunen, E.: Wirkungen der Luftzufuhr auf aromatische Verbindungen bei der immobilisierten Hauptgärung — Monatsschrift für Brauwissenschaft 52, Nr. 1/2, 9 – 12, 25 – 28, 1999

BC 2 Malz- und Bierbereitung/ 23 Gärung

Die kontinuierliche immobilisierte Hauptgärung ist seit dem vergangenen Jahrhundert Gegenstand von Untersuchungen, hat aber bislang die in sie gesetzten Erwartungen nicht erfüllt. Bei der kontinuierlichen Hauptgärung gibt es zwei Hauptschwierigkeiten biologischer Art: Erreichen und Erhaltung des gewünschten Aromas und Verhinderung von Kontaminationen. Ein herkömmliches Verfahren zur Kontrolle des Aromas von Bier besteht in der Belüftung der Bierwürze vor dem Anstellen. Wir haben die Wirkung der Luftzufuhr auf die immobilisierte Hauptgärung untersucht. Das verwendete Fermentationssystem bestand aus zwei Festbettreaktoren und einem Puffertank zwischen den Reaktoren. Es wurde industrielle Bierwürze und ein Hefestamm einer gewerblichen Brauerei verwendet. Die Belüftung der Bierwürze erfolgte durch Einleitung von Luft in den Bierwürzestrom kurz vor dem Einlaß des ersten Reaktors (Vorkolonne). Die Luft wurde mit Kohlendioxid verdünnt, und beides wurde durch zwei Membrane mit 0,2 µm aus PTFE (Domnick Hunter Ltd., Durham, Vereinigtes Königreich) gefiltert. Die Zufuhrmengen der synthetischen Luft und des Kohlendioxids wurden unabhängig voneinander nach einem experimentellen Entwurf nach Box-Hunter geändert, während die Zufuhrmenge der Bierwürze konstant gehalten wurde. Die Ergebnisse wurden mit Hilfe des Computerprogramms für experimentelles Konstruieren, Modde 3.0 (Umetri AB, Uppsala, Schweden), analysiert, und es wurden mathematische Modelle für die Konzentrationen höherer Alkohole und zweier Essigsäureester im Auslauf der Vorkolonne erstellt. Es stellte sich heraus, daß sowohl geringe Luftzufuhr als auch hohe Luftzufuhr bei geringer Zufuhr von Kohlendioxid die Produktion von 3-Methylbutylazetat in der Vorkolonne förderte. Die Hauptkolonne schien die Veränderungen der analysierten aromatischen Verbindungen auszugleichen, so daß ihre jeweilige Endkonzentration innerhalb des untersuchten Bereichs durch Änderungen der Luftzufuhr relativ schwach beeinflußt wurde.

Virkajärvi, I., Lindborg, K., Kronlöf, J., et Pajunen, E.: Actions de l'apport d'air sur les composés aromatiques au cours de la fermentation primaire immobilisée — Monatsschrift für Brauwissenschaft 52, Nr. 1/2, 9 – 12, 25 – 28, 1999

BC 2 Fabrication du malt et de la bière/ 23 Fermentation

La fermentation primaire en continu immobilisée fait l'objet d'investigations depuis le siècle dernier, mais n'a pas répondu aux espérances fixées. Deux principales difficultés de nature biologique sont rencontrées pour la fermentation primaire en continu: obtenir et maintenir l'arôme souhaité et empêcher les contaminations. Un procédé traditionnel pour le contrôle de l'arôme de la bière réside dans l'aération du moût de bière à l'entonnement. Nous avons examiné l'action de l'apport d'air sur la fermentation primaire immobilisée. Le système de fermentation utilisé était composé de deux réacteurs de fermentation à lit fixe et un tank tampon entre les deux réacteurs. On a utilisé du moût de bière industriel et une souche de levure d'une brasserie industrielle. L'aération du moût de bière a été réalisé par l'injection d'air dans le circuit du moût de bière juste avant l'entrée du premier réacteur (pré-colonne). L'air a été dilué avec de l'anhydride carbonique et les deux ont été filtrés sur deux membranes de 0,2 µm en PTFE (Domnick Hunter Ltd., Royaume Uni). L'apport quantitatif de l'air synthétique et de l'anhydride carbonique a été varié de façon indépendante suivant un plan expérimental d'après Box-Hunter, pendant que l'apport du moût de bière a été maintenu constant. Les résultats ont été analysés à l'aide d'un programme informatique de constructions expérimentales, modèle 3.0 (Umetri AB, Uppsala, Suède) et on a établi des modèles mathématiques pour la concentration d'alcools supérieurs et de deux esters de l'acide acétique à la sortie de la pré-colonne. Il s'est avéré que la production de l'acétate 3-méthylbutyle au niveau de la pré-colonne était augmenté aussi bien par l'apport d'air faible, l'apport d'air important avec un faible apport d'anhydride carbonique. La colonne principale semble équilibrer les changements des composés aromatiques analysés de telle sorte que la concentration individuelle finalereste à l'intérieur du domaine analysé et n'est que relativement peu influencée par l'apport d'air.

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International Subcommittee for Isomerised Hop α-Acids Standards

The International Subcommittee for Isomerised Hop α-acids Standards held its inaugural meeting at the Hilton and Towers Hotel in Minneapolis on 21st September 1998. Nine members of the committee or their nominated representatives and four guests attended the meeting, which included persons from Europe, the United States of America and Australia.

This newly formed committee has been charged with the task of organising the selection, validation, manufacture and distribution of International Standards for the analysis of isomerised and reduced isomerised α-acids (specifically, iso-α-acids, rho-iso-α-acids, tetrahydroiso-α-acids and hexahydroiso-α-acids). It is pioneering a new approach to international collaboration on analytical methodology and has a current membership comprising a chairman plus six officially nominated representatives from ASBC, three from EBC, two from IOB and one from BCOJ (the Brewery Convention of Japan). Brewers, hop processors and research organisations are all well represented:

Dr. Richard Wilson (Steiner Hops, Chairman)

Dr. Robert Foster (Coors Brewing, Vice Chairman, Americas)

Dr. Martin Biendl (Hallertauer Hopfenveredelungs GmbH, Vice Chairman, Rest of World)

Mr. Roy Cope (Bass Brewers)
Dr. Paul Hughes (Brewing Research International)
Dr. Heini Pfenninger (Versuchsstation Schweizerischer Brauereien)
Mr. Leen Verhagen (Heineken)
Mr. Shuso Sakuma (Kirin)
Dr. James Guzinski (Kalsec)
Mr. James Murphey (Murphey Analytical Laboratories)
Dr. John Paul Maye (Haas Hop Products)
Mr. Robert Smith (S. S. Steiner)
Dr. Patrick Ting (Miller Brewing)

Following introductory remarks from the Chairman and agreement as to constitutional aspects of how this committee will function there followed discussion of the issues to be addressed and the way in which these will be tackled. Such questions included: "Who will manufacture the new standards?"; "From what starting materials?"; "How will the purity and stability be checked?"; "Do we even need different standards for each type of isomerised compound?".

After debating these and other such issues, the committee agreed upon the following course of action:

Several laboratories will prepare 2 – 4 gm of any or all of the following substances:

1. DCHA – Iso-α-acids (Dicyclohexylamine salt of iso-α-acids)
2. DCHA – Rho-Iso-α-acids
3. Tetrahydroiso-α-acids (as crystalline, free acids)
4. DCHA – Hexahydroiso-α-acids

These compounds will be prepared using the methods of Dr. John Paul Maye, shortly to be published in the Journal of the ASBC. The different laboratories will be free to choose any commercially available isomerised or reduced isomerised extract as their base material. In the case of the putative "Tetra" and "Hexa" standards, deliberately different preparations will be made to reflect the fact that commercially available base products may originate from either α- or β-acids and that these have markedly different cohumulone ratios.

It is intended that this preparative work will have been completed by mid-January, 1999. The committee will then decide upon the means by which the various purified compounds will be analysed and otherwise assessed for suitability as standards. At this meeting, four hop processors and one research organisation indicated their willingness to undertake some at least of the necessary syntheses and are to be thanked for their enthusiastic co-operation: John I. Haas, Inc.; Kalsec, Inc.; S. S. Steiner, Inc.; Wigan Products Ltd. and Brewing Research International. Any other organisation that would like to join in this exercise is cordially invited to do so and should contact the Chairman or the Vice-Chairman for their area as soon as possible.

An invitation is also extended to all individuals who feel that they could contribute to the work of the committee to make contact with one of its officers. During the course of the meeting it was agreed to create a category of "Associate Members" for the specific purpose of encouraging such participation. In this way, it is hoped to ensure that all interested persons are given the opportunity to make their views known and to assist the committee in the undertaking of such tasks as ring analyses.

Dr. Richard J. H. Wilson, Chairman