STATE OF THE ART OF IMMOBILISED CELL TECHNOLOGY FOR BEER BREWING

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Traditional beer fermentation technology uses freely suspended yeast cells to ferment wort in a non–stirred batch reactor. These fermentations are very time consuming. The primary fermentation for lager beer takes approximately 7 days with a subsequent secondary fermentation (maturation) of several weeks. However, the resulting beer has a well–balanced flavour profile which is very well accepted by the consumer. Modern batch fermentation technology can reduce the production time (main and secondary fermenation) of lager beer to 10 – 12 days.

Immobilised cell technology (ICT) is able to produce lager beer in less than 2 days. The bottleneck is to achieve a correct balance of sensory compounds to create an acceptable flavour profile in such a short time frame. ICT for beer production can only be introduced successfully if the flavour profile can be controlled and fine tuned. Therefore, a fully understanding of the metabolic behaviour of the immobilised cells is necessary. The physiology of immobilized cells can substantially deviate from free cell physiology due to mass transfer limitations. Matrix and reactor design can have an important impact on mass transfer and thus on cell physiology (Masschelein et al., 1994; Norton et al., 1994; Baron et al., 1996; Willaert et al., 1990; Masschelein, 1997; Pilkington et al., 1998).

ICT PROCESSES FOR THE PRODUCTION OF BEER

ICT processes have been designed for different stages in the beer production/fermentation process: wort acidification, bioflavouring during the secondary fermentation, main fermentation, fermentations for the production of alcohol–free or low–alcohol beers. The most challenging and most complicated application is the combined main and secondary fermentation. In this paragraph, some examples of ICT production processes during the different production steps are discussed.

Wort acidification

The pH of the wort after extraction is too high to start the boiling process. An adjustment of the wort pH is necessary to have a good hot break formation, α – acid isomerisation and a less intense Maillard reaction. Brewing according to the "Reinheitsgebot" (Germany) does not allow the use of adding acids to the wort. Instead, a fermentation of the wort with lactic acid bacteria can be used to acidify the wort. Characteristics of an industrial ICT process is tabulated in Table 1.

Green beer maturation

The main objective of flavour maturation (or secondary fermentation) is the removal of the vicinal diketones diacetyl and 2,3–pentanedione, and their precursors α–acetolactate and α–acetohydroxybutyrate. Vicinal diketones are formed by an oxidative decarboxylation from the excess α–acetohydroxy acids which leak from the isoleucine–valine pathway. Diacetyl is reduced by yeast reductases to 2,3–butanediol *via* acetoin and 2,3–pentanedione to 2,3–pentanediol *via* acethylethylcarbinol. The conversion of the α–acetohydroxy acids to the vicinal diketones is the rate limiting step. This reaction step is accelerated by heating the beer after yeast separation (using a centrifuge) to 80–90°C during a few minutes. The resulting vicinal diketones can subsequently be reduced by immobilised yeast cells.

The traditional maturation process is characterised by a near–zero temperature, low pH and low yeast concentration, resulting in a maturation period of 3 to 4 weeks. ICT can reduce this period to 2 hours. Examples of ICT maturation processes are illustrated in Table 2.

It should also be mentioned that accelerated beer maturation can also be performed by increasing the maturation temperature and pH reduction (McMurrough, 1995). This can be realised by integrating the secondary fermentation in the primary fermentation. In this way, the production of beer can be accomplished in less than 1 week in one cylindroconical vessel. This new fermentation technology can be used as cost effective as ICT.

Alcohol–free or low–alcohol beer fermentation

The traditional technology to produce alcohol–free or low–alcohol beer is based on the suppression of alcohol formation by arrested (restricted) batch fermentation (Muller, 1990; Narziss et al., 1992). However, the resulting beers are characterised by an undesirable wort aroma since the wort aldehydes have

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Table 1. ICT for wort acidification.

Immobilisation Method	Immobilisation Matrix	Micro–organism	Reactor Type	Scale	Reference
Surface attachment	DEAE–cellulose	*Lactobacillus amylovorus*	PBR[a]	Industrial	Pittner et al., 1993; Meersman, 1994

[a]Packed–bed reactor

Table 2. ICT for beer maturation.

Immobilisation Method	Immobilisation Matrix	Reactor Type	Scale	Reference
Surface attachment	DEAE–cellulose beads	PBR	Industrial ($1{\cdot}10^6$ hl/year)	Pajunen, 1995
Entrapment	Porous glass beads	PBR	Industrial ($4{\cdot}10^5$ hl/year)	Dillenhöfer, 1996; Mensour et al., 1997; Back et al., 1998

Table 3. ICT for the production of alcohol–free or low–alcohol beer.

Immobilisation Method	Immobilisation Matrix	Reactor Type	Scale	Reference
Surface attachment	DEAE–cellulose	PBR	Laboratory	Colin et al., 1991; Van Dieren, 1995
Surface attachment	DEAE–cellulose	PBR	Industrial	Pittner et al., 1993; Mensour et al., 1997
Entrapment	Porous glass	Fluidised bed	Pilot	Aivasidis et al., 1991; Breitenbücher et al., 1995; Aivasidis, 1996
Entrapment	Silicon carbide rods	Cartridge loop Reactor	Pilot	Van De Winkel et al., 1995
Entrapment	Ca–pectate	PBR	Lab	Kaclìková et al., 1992; Mockovciaková et al., 1993; Navrátil et al., 2000

Table 4. ICT for the main fermentation.

Immobilisation Method	Immobilisation Matrix	Reactor Type	Reference
Entrapment	Ca–alginate	Airlift	Nedovic et al., 1996, 1997a, 1997b
Entrapment	Ca–alginate beads Ceramic beads	Stirred tank[a]+2 PBR[b]	Inoue, 1995; Yamauchi et al., 1994; Ceramic beads Yamauchi et al., 1995
Entrapment	Silicon carbide rods	Cartridge loop reactor[b] + stirred tank[a]	Andries et al., 1996, 2000; Masschelein and Andries, 1995
Entrapment	κ – carrageenan beads	Airlift	Mensour et al., 1995, 1996, 1997
Adsorption	Woodchips	PBR	Kronlöf et al., 1999
Adsorption	DEAE–cellulose	PBR	Andersen et al., 1999
Adsorption	Spent grains	Airlift	Brányik et al., 2002

[a]Free cells; [b]Immobilised cells

only been reduced to a limited degree (Collin et al., 1991; Debourg et al., 1994; van Iersel et al., 2000). The reduction of these wort aldehydes can be quickly achieved by a short–contact with the immobilised yeast cells at a low temperature without undesirable cell growth and ethanol production. A disadvantage of this short contact process is the production of only a small amount of desirable esters.

Another method of producing these beers is based on the removal of ethanol from stronger beers by using membrane, destillation or vacuum evaporation processes (Huige et al., 1990).

Controlled ethanol production for low–alcohol and alcohol–free beers have been successfully achieved by partial fermentation using immobilised yeast (Table 3).

Main fermentation

The design and optimisation of an ICT process for the combined main and secondary fermentation remains a challenging task, although encouraging result have been obtained on lab and pilot scale during the last years (Table 4). The reasons why these ICT processes have not yet been adopted in the brewing industry include complexity of operations compared to batch processes, flavour problems (due to a lack of understanding and controllability of the changed metabolism), yeast viability and carrier price. Especially the altered metabolism and the knowledge to tune the metabolism to the desired flavour needs to be further investigated in the near future (Ryder, 2002; Shen et al., 2003a,b).

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