Modelling and design of an immobilized cell process for solvent production

Proefschrift ter verkrijging van de graad van doctor in de technische wetenschappen aan de Technische Hogeschool Delft, op gezag van de Rector Magnificus, Prof. Dr. J. M. Dirken, in het openbaar te verdedigen ten overstaan van het College van Dekanen op donderdag 15 mei 1986 te 14.00 uur door
GEERTRUIDA HELENA SCHOUTENS
geboren te Woensdrecht
doctorandus scheikunde.
Dit proefschrift is goedgekeurd door de promotoren

Prof. dr. ir. N.W.F. Kossen
Prof. ir. K. Ch. A. M. Luyben

I would like to pay special tribute and offer thanks to my father-in-law, Anton Poot, for kindly arranging the printing and publication of this thesis.
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Like many biotechnological studies, this thesis treats a broad spectrum of subjects, all of which are aspects of one central theme. This central theme is the use of immobilized micro-organisms in bioreactors, particularly the mathematical modelling and process design of a fermentation with immobilized cells.

More specifically, this thesis describes the influence of immobilization on the kinetics of micro-organisms, mass transport phenomena involved and the behaviour of the biocatalyst particles in three phase flow bioreactor systems. All these aspects were applied to and carried out within the framework of the butanolic fermentation by Clostridium-bacteria. In this fermentation, Clostridia produce the commercially interesting butanol, as well as isopropanol (or acetone) and fermentation gas, from various carbohydrate sources.

The energy problem of the seventies can be seen as the main driving force behind the revival of interest in fermentations producing solvents and fuels from renewable resources\(^1\); this led and is still leading to a large number of groups performing research in this area.

Table 1 gives an impression of research results published in 1985 on the butanolic fermentation with Clostridia (own work not included). It may be clear that this fermentation is being looked at from totally different angles by a large (and still increasing) number of research groups.

Two approaches can be distinguished. The first one can be called a microbiological/mechanistic approach, aimed at elucidation of the genetics, metabolism, physiology etc., to obtain a better understanding of and a firmer grip on the micro-organisms of interest (i.e. Clostridia). Eventually this will lead to the long awaited mutant strains with high butanol tolerances\(^1,6,7\).
Table 1: Some research results published in 1985 on the butanolic fermentation.

<table>
<thead>
<tr>
<th>Effect of........</th>
<th>....on........</th>
<th>....studied by........</th>
</tr>
</thead>
<tbody>
<tr>
<td>recombination</td>
<td>Cl.acetobutylicum P262</td>
<td>Woods, Jones3(South Africa)</td>
</tr>
<tr>
<td>CO</td>
<td>metabolic pathways</td>
<td>Datta, Zeikus4(U.S.A.)</td>
</tr>
<tr>
<td>pressure, agitation</td>
<td>solvent production</td>
<td>Doremus, Moreira5(U.S.A.)</td>
</tr>
<tr>
<td>products</td>
<td>metabolism and sugar uptake</td>
<td>Bowles, Ellefson6(U.S.A.)</td>
</tr>
<tr>
<td>products</td>
<td>metabolism of mutants sugar uptake rate</td>
<td>Vandecasteele7(France)</td>
</tr>
<tr>
<td>products, substrate</td>
<td>solvent production</td>
<td>Ounine, Gay8(France)</td>
</tr>
<tr>
<td>temperature</td>
<td>solvent production, growth</td>
<td>Fond, Engasser9(France)</td>
</tr>
<tr>
<td>strain used</td>
<td>solvents from whey p.</td>
<td>McNeill, Kristianse10(U.K.)</td>
</tr>
<tr>
<td>strain used</td>
<td>solvents from xylene production from lignocellulose</td>
<td>Ennis, Maddox11(New Zealand)</td>
</tr>
<tr>
<td>e-culturing</td>
<td>solvents from whey p.</td>
<td>Wayman, Yu12(Canada)</td>
</tr>
<tr>
<td>temperature</td>
<td>continuous solvent production</td>
<td>Yu, Chan, Saddler13(Canada)</td>
</tr>
<tr>
<td>cell recycling</td>
<td>cont. solvent prod.</td>
<td>Vogler, Ertol14(Argentina)</td>
</tr>
<tr>
<td>dilution rate</td>
<td>cont. solvent prod.</td>
<td>Afshari, Bieli15(W-Germany)</td>
</tr>
<tr>
<td>substrate, nutr.</td>
<td>cont. solvent prod.</td>
<td>Fick, Engasser16(France)</td>
</tr>
<tr>
<td>cell adsorption</td>
<td>cont. solvent prod.</td>
<td>Stephens, Morris17(U.K.)</td>
</tr>
<tr>
<td>immobilization</td>
<td>solvent production by mutants</td>
<td>Föhrberg, Högström18(Sweden)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jones, Woods19(South Africa)</td>
</tr>
</tbody>
</table>

The second approach, of which the work presented here is an example, can be seen as a chemical engineering/technological one, aimed at the development of fermentation modes, reactors, suitable carbohydrate sources and product recovery methods to optimize the process as a whole. A combination of the two approaches is highly recommended, but rarely encountered, probably due to the diversity of disciplines required.

This thesis consists of 9 separate papers, which have already been published or are still in press. The papers can be read independently, some repetitions and overlaps do occur and were inevitable. Each paper treats a different aspect of the design of the continuous butanol production process by immobilized Clostridia.

The first two papers deal with the optimization of substrate and process conditions. PAPER I reports on the conditions Cl.beyernickii requires to produce butanol/isopropanol from the substrate whey permeate. The results were obtained from free cell batch fermentations and directly led to the study described in the subsequent paper.

PAPER II describes the immobilization of Cl.beyernickii in calcium alginate beads. The conditions necessary for continuous butanol/isopropanol production from whey permeate with these immobilized cells are presented. These results are compared with Krouwel's1 results on glucose.

Having established the conditions for reproducible butanol production from whey permeate, the kinetic modelling of the immobilized cell system was carried out.

PAPER III describes the development of a kinetic model and the application to immobilized Cl.beyernickii on glucose. Meanwhile the constant search for other "immobilizable", productive strains led to an undefined strain, Cl.species DSM 2152. The testing of the kinetic model with this strain in an immobilized form is reported in PAPER IV. Furthermore, the application of the kinetic model is extended to whey permeate substrates.

PAPER V forms the introduction to the reactor design study. Two reactor types suitable for a large scale use of gas producing, immobilized cells were selected, designed on production scale from literature data and estimations and scaled down to laboratory scale.

PAPER VI, consecutively, treats the hydrodynamic modelling of these three phase flow reactors. With the kinetic model of papers III and IV a combined model for the total reactor performance is set up.
PAPER VII, ultimately, describes the continuous butanol production with immobilized Clostridia in these reactors and the consistency of the results with the model predictions. Concludingly, scaling up of the model reactors is commented upon.

PAPER VIII forms a preliminary economic feasibility study of the IBE process: the continuous production of butanol/isopropanol mixtures with immobilized Clostridia from whey permeate on a large scale. The paper has two main authors; the product recovery section is written by W. Groot, working on the research project 'simultaneous product recovery in product inhibited fermentations'.

PAPER IX forms an extension of paper VIII; it is a short communication on the application of butanol/isopropanol mixtures as engine fuels. Table 2 presents a schematic summary of the papers.

Table 2: Schematic summary of the papers.

<table>
<thead>
<tr>
<th>PAPER</th>
<th>AIM</th>
<th>CONDITIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>fermentation conditions</td>
<td>substrate: whey p.</td>
</tr>
<tr>
<td>II</td>
<td>fermentation conditions</td>
<td>substrate: whey p.</td>
</tr>
<tr>
<td>III</td>
<td>macrokinetic model development</td>
<td>substrate: glucose</td>
</tr>
<tr>
<td>IV</td>
<td>macrokinetic model extension</td>
<td>substrate: glucose</td>
</tr>
<tr>
<td>V</td>
<td>reactor design/ scale down</td>
<td>substrate: glucose</td>
</tr>
<tr>
<td>VI</td>
<td>hydrodynamic model/ integrated model</td>
<td>substrate: glucose</td>
</tr>
<tr>
<td>VII</td>
<td>integrated model test/ scale up</td>
<td>substrate: glucose</td>
</tr>
<tr>
<td>VIII</td>
<td>economic feasibility</td>
<td>substrate: whey p.</td>
</tr>
<tr>
<td>IX</td>
<td>product application/ engine tests</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1 schematically shows the structure of this thesis and the relation between the various papers.

A concluding chapter was not added to the thesis as it was thought each paper with its own introduction and concluding section was self-explanatory. The general introduction and summary depict both framework and consistency of the thesis.

Figure 1: Schematic arrangement of aspects treated in this thesis.
REFERENCES


The central theme of this thesis is the use of immobilized cells in bioreactors; the fermentation of sugars to butanol/isopropanol mixtures by Clostridia forms the framework for this central theme. The thesis is closely related to P.G.Krouwel's thesis (see Introduction), in which it was demonstrated that Clostridia, when immobilized, are able to produce butanol and isopropanol continuously from the model substrate glucose.

The first part of the thesis (papers I and II) concerns fermentations of a technical substrate, whey permeate, which is a by-product of cheese manufacture. It reports the proper fermentation conditions to produce butanol/isopropanol mixtures from whey permeate with Clostridium beyerinckii LMD 27.6. It is noteworthy that both batch fermentations with free cells and continuous fermentations with immobilized cells need to be carried out at 30°C (as opposed to 37°C for glucose). Furthermore, it appears that a low dilution rate (less than 0.1 hr⁻¹) during the start-up phase of the continuous fermentation is of vital importance to a favourable development of biomass within the immobilization matrix (calcium alginate). The butanol and isopropanol production rates for whey permeate fermentations at 30°C were determined to be a factor 2-3 lower than for glucose fermentations at 37°C; as yet it seems this cannot be explained by the difference in temperature.

Next the thesis deals with the development of a simple model to describe the macrokinetics of immobilized Clostridia (papers III and IV). The model is first developed for (immobilized) Cl. beyerinckii on the model substrate glucose. In this derivation it is essential that:

- linear butanol inhibition kinetics describes the experimental results satisfactorily
- the biomass in the calcium alginate matrix appears to be distributed in colonies throughout the entire spherical particle

An inhibiting butanol concentration, C_BM*, of 5.5 kg/m³ was found and a maximal specific conversion rate of 50 kg/m³ alginate.hr.
With this model the continuous production of butanol and isopropanol is predicted well as a function of the dilution rate, the reactor loading (fraction biocatalyst) and, implicitly, the substrate feed concentration. After this the model has been applied to another butanol producing strain, *Clostridium* DSM 2152 (again immobilized in calcium alginate) and to the technical substrate, whey permeate. This leads to a number of important conclusions:

- The fermentation of whey permeate does not differ substantially from the fermentation of glucose; a stronger inhibition of butanol on lactose containing substrates (C_{BH} = 4.7 kg/m^3) in comparison with glucose (C_{DH} = 7.4 kg/m^3) explains the lower production rate on whey permeate noted previously.
- The same simple kinetic model can be used for *Clostridium* on glucose as well as on whey permeate (r_{max} = 55 kg/m^2alginate.hr).
- *Clostridium*, when immobilized, forms an extremely stable biocatalyst. Continuous experiments of 1000-1800 hours are no exception.

The study of reactor types suitable for this process forms the next phase (papers V, VI, VII). Based on the demands of the immobilized and gas producing system upon the reactor (e.g. with respect to mixing, attrition, gas release), a choice is made for two three-phase reactors, applicable on an industrial scale: a fluidized bed reactor with liquid recycle and an external loop gas lift reactor. On the one hand this study was carried out to obtain more information on the still scarcely described, but in biotechnology more often applied subject of three phase systems. On the other hand these reactors were tested and compared with respect to their applicability in the IBE process.

For both reactors a laboratory scale design (10-15 l) is made on the basis of a theoretical design for industrial scale, using an evaluation of the time constants of the most important processes in those reactors. A model is composed and tested to describe the mixing in these reactors, leading to a plug flow with recycle model for the fluidized bed reactor and a stirred tank model for the gas lift loop reactor. Combined with the kinetic model for immobilized *Clostridium* this leads to a general reactor model.

In conclusion continuous fermentations are carried out in the laboratory scale reactors, leading to a satisfactory performance of the general reactor model (Productivities of 1.5-3.0 kg butanol-isopropanol/m^3.hr).

Important to notice is the robustness of the process, particularly in the fluidized bed reactor, as well as the relative insensitivity to inclusions and oxygen. Furthermore the fluidized bed reactor exhibits a regular fluidization, which can be described by a two phase (liquids-solids) model. The third phase -gas- moves undisturbed through the bed in small, hardly coalescing bubbles (0.5-1.0*10^-3 m diameter). The conditions are indicated for which the developed reactor models can be used to draw conclusions with respect to fermentation productivity and the behaviour of the three phase system in these reactors on production scale. On the basis of a comparison of the two reactors (on e.g. productivity, possibility for simultaneous recovery) a fluidized bed reactor with liquid recycle is preferred for application in the industrial butanol-isopropanol production with immobilized *Clostridium*.

Parallel to the previously discussed studies a preliminary cost estimate study was carried out in cooperation with W.J. Groot (papers VIII and IX). In this study the continuous production of butanol/isopropanol mixtures (by an immobilized *Clostridium* strain) is combined with a simultaneous recovery of the products (via a membrane process). The high production rates of the immobilized system is further increased by removal of the inhibiting product butanol. On the basis of some laboratory scale results, combined with literature estimations it is indicated that under certain conditions this process, using whey permeate as a substrate and forming butanol/isopropanol as products, can become economically feasible. Whether such a process will be applied on an industrial scale depends to a large extent on the (regional) developments of the oil price determined butanol/isopropanol market. To a considerable extent the results in this thesis, reported particularly on the central theme, the use of immobilized cells in bioreactors, can be applied directly in the field of similar biotechnological processes.
Het gebruik van geimmobiliseerde cellen in bioreaktoren is het centrale thema van dit proefschrift; de fermentatie van suikers tot butanol/isopropanol mengsels door Clostridium-bacteriën het kader waarbinnen het onderzoek heeft plaatsgevonden.

Het proefschrift sluit aan op dat van P.G.Krouwel (Zie Introduction), waarin ondermeer is aangetoond dat Clostridia in geimmobiliseerde vorm continu butanol en isopropanol kunnen maken uit het modelsubstraat glukose.

Het eerste deel van het proefschrift (artikelen I en II) is gewijd aan het verkrijgen van de juiste fermentatie condities om uit een technisch substraat, wei-permeaat (laktose houdend bijprodukt kaasfabrikage), butanol/isopropanol te produceren met Clostridium beyerlincil LMD 27.6.

Opmerkelijk hierbij is dat zowel batch fermentaties met vrije cellen als continue fermentaties met geimmobiliseerde cellen bij 30°C (in tegenstelling tot 37°C voor glukose) uitgevoerd dienen te worden. Voorts blijkt dat een lage verdunningssnelheid (kleiner dan 0.1 hr⁻¹) tijdens de startfase van de continue fermentatie van vitaal belang is voor een goede ontwikkeling van biomassa in de immobilisatiematrix (calciumalginaat).

De gevonden butanol en isopropanol-productiesnelheden voor de wei-permeaat fermentaties bij 30°C blijken een factor 2 tot 3 lager te liggen dan voor de glukose fermentaties bij 37°C. Vooral nog lijkt dit verschil bij lange na niet door het temperatuursverschil verklaard te kunnen worden.

Vervolgens behandelde het proefschrift de ontwikkeling van een eenvoudig model om de makrokinetiek van geimmobiliseerde Clostridia te beschrijven (artikelen III en IV). In eerste instantie wordt dit model voor Cl. beyerlincil (geimmobiliseerd) op het modelsubstraat glukose afgeleid.

Essentieel hierbij is dat:
- een lineaire butanol inhibitie kinetiek de experimenten goed beschrijft
- de biomassa in de calciumalginaat matrix in kolonievorm door het gehele bolvormige deeltje verspreid blijkt

Uiteindelijk is een remmende butanolconcentratie, $C_{RM}$, van 5.5 kg/m³ en een maximale specifieke konversiesnelheid, $r_{max}$, van 50 kg/m³•alginaat.hr
Gevonden.

Met dit model wordt de continue produktie van butanol/isopropanol als functie van de verdunningssnelheid, de reaktorbelading (fraktie biokatalysator) en impliciet, de substraatconcentratie goed voorspeld. Hierna is het model toegepast op een andere butanolproducerende stam, Cl. species DSM 2152 (wederom geimmobiliseerd in calciumalginaat) en op het technische substraat wei-permeaat. Dit leidt tot een aantal belangrijke conclusies:

* de fermentatie van wei-permeaat is niet essentieel verschillend van de fermentatie van glukose; een gebleken sterkere remming van butanol op de conversie van lactose houdende substraten (C =4.7 kg/m$^3$), in vergelijking met glukose (C$_{BM}$=7.4 kg/m$^3$), verklaart de al eerder opgemerkte lagere produktiesnelheid op wei-permeaat

* hetzelfde eenvoudige kinetiekmodel is bruikbaar voor Cl. species op zowel glukose als wei-permeaat (r$_{max}$=55 kg/m$^3$ alginaat.hr)

* Cl. species is in geimmobiliseerde vorm een extreem stabiele biokatalysator; continue experimenten van 1000-1800 uur vormen geen uitzondering

Een volgende fase vormt de bestudering van reaktortypen, geschikt voor dit proces (artikelen V, VI, VII). Hierbij is, uitgaande van de eisen die het geimmobiliseerde en gasproducerende systeem aan de reaktor stelt (vb. ten aanzien van menging, attritie en gasafvoer), een keus gemaakt voor een tweetal op industriële schaal bruikbare driefasen reactoren: een geëvaporiseerd bed reaktor met vloeistof recirculatie en een externe luchtloop reaktor. Enerzijds is deze studie verricht om meer inzicht te verkrijgen in het nog weinig beschreven, maar in de biotechnologie steeds vaker toegepaste onderwerp van driefase systemen. Anderzijds zijn deze reactoren getest en vergeleken op hun toepasbaarheid in het butanol/isopropanol proces. Voor beide reactoren is, aan de hand van een theoretisch ontwerp op industriële schaal, een ontwerp op laboratoriumschaal (10-15 l.) gemaakt, op basis van een tijdkonstante-évalueatie van de belangrijkste deelprocessen in die reaktoren. Er is een model opgesteld en getest voor de menging in deze reaktoren, wat leidt tot een plug flow met recycle model voor de geëvaporiseerd bed reaktor en een geroerde tank model voor de gas lucht loop reaktor.

Gecombineerd met het kinetiekmodel voor geimmobiliseerde Clostridia leidt dit tot een overall reaktormodel.

Tenslotte zijn continue fermentaties in de laboratoriumschaal reactoren uitgevoerd, waaruit blijkt dat de reaktormodellen goed voldoen. (Productiviteiten van 1.5-3.0 kg/18 m$^3$.hr). Opmerkelijk hierbij is dat met name in de geëvaporiseerd bed reaktor het proces uiterst robuust en weinig gevoelig voor infekties en zuurstof is. Tevens blijkt de geëvaporiseerd bed reaktor een zeer regelmatige fluidisatie te vertonen, die zich met een tweefasen model (L-S) goed laat beschrijven. De derde fase (gas) beweegt zich in de vorm van kleine belletjes (0.5-1.0*10$^{-3}$ m diameter) nauwelijks coalescerend en ongestoord door het reaktorbed.

Onderzoek is binnen welke randvoorwaarden de ontwikkelde reaktormodellen bruikbaar zijn om uitspraken te doen over fermentatieproductiviteit en het gedrag van de driefase systemen in deze reaktoren op productieschaal. Op grond van een vergelijking van de twee reactoren (o.a. op basis van produktiviteit en mogelijkheid voor simultane opwerking) wordt de voorkeur gegeven aan een geïmmobiliseerd bed reaktor met vloeistof recirculatie voor toepassing in de industriële butanol/isopropanol produktie met geimmobiliseerde Clostridia.

Het laatste deel van het proefschrift behandelt een voorlopige kostprijzenstudie, die is uitgevoerd parallel aan de eerder besproken studies. Deze kostprijzenstudie is verricht in samenwerking met W. Groot (artikelen VIII en IX). In deze studie is de continue produktie van butanol/isopropanol (door een geimmobiliseerde Clostridium stam) gecombineerd met een simultane opwerking van de produkten (via een membraanproces). De hoge produktiesnelheid van het geëvaporiseerde systeem wordt nog eens extra verhoogd door afvang van het remmende produkt butanol. Uitgaande van enige laboratoriumschaal resultaten, literatuurgegevens en schattingen is aan te geven, dat binnen bepaalde voorwaarden dit proces, met wei-permeaat als grondstof en butanol/isopropanol als produkt, economisch rendabel kan worden. Of een dergelijk proces op industriële schaal zal worden toegepast, hangt in hoge mate af van de (regionale)ontwikkelingen op de door olieprijzen beheerste butanol/isopropanol markt. Voor een niet-onaanstelbaar deel kunnen de resultaten in dit proefschrift die met name betrekking hebben op het centrale thema, het gebruik van geimmobiliseerde cellen in bioreaktoren, direct toegepast worden in het kader van soortgelijke biotechnologische processen.
Butanol from whey ultrafiltrate: batch experiments with Clostridium beyerinckii LMD 27.6.

Butanol from whey ultrafiltrate: batch experiments with Clostridium beyerinckii LMD 27.6

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Summary. For batch fermentations by Clostridium beyerinckii LMD 27.7 (formerly known as Clostridium butylicum) whey ultrafiltrate, glucose, lactose, and galactose were used as substrates. The aims of the experiments were to find the conditions for butanol production from whey ultrafiltrate and to compare the results with those of other substrates. The conditions necessary for butanol production were established. The mean solvent productivity and thus to lower the cost of these immobilized cells yielded butanol and isopropanol continuously in reactor productivity six to 16 times higher than that found on glucose; the overall solvent yields were comparable. Butanol production from galactose and mixtures of glucose and galactose was also possible.

Introduction
The renewed interest in fermentative solvent production is clearly revealed by the number of recent publications on the subject (Chua et al. 1980; McGhee et al. 1982; Cheetham 1980). New technology is used to improve reactor productivities and thus to lower the cost of these fermentatively produced solvents. Attempts are made to produce the solvents continuously and for some applications immobilization of the solvent-producing cells offers possibilities (Häggström et al. 1980; Häggström 1981).

For the butanol-isopropanol production by immobilized Clostridium beyerinckii cells promising results have already been reported (Krouwel 1982; Krouwel et al. 1983). Krouwel found that on glucose media these immobilized cells yielded butanol and isopropanol continuously in reactor productivity six to 16 times higher than in batch cultures of free cells. One of these areas in which the results of Krouwel could be improved was the choice of substrate; the possibilities of replacing glucose by a cheaper, industrially more interesting substrate needed to be investigated.

When whey ultrafiltrate, a by-product of cheese manufacture, could be a possible substrate for this fermentation (Lenz et al. 1980). It consists of lactose, a disaccharide formed by glucose and galactose. In many countries whey forms a serious waste-disposal problem. However, before continuous experiments with whey ultrafiltrate and immobilized cells could be started, more knowledge was required about the behaviour of Cl. beyerinckii in lactose-containing media; in the literature there is very little information (Gapes et al. 1982; Maddox 1980).

Batch experiments with Cl. beyerinckii LMD 27.6 were carried out to find the process conditions needed for the production of butanol from whey ultrafiltrate. Variations in medium composition, fermentation temperature, inoculum pretreatment and pH were considered. The results of these experiments are reported here.

Materials and methods
Micro-organisms. The anaerobic spore-former Cl. beyerinckii LMD 27.6 was obtained from the Laboratory of Microbiology, Delft University of Technology. The organism was cultured on a medium of 60 kg/m³ glucose (Baker, The Netherlands), 13 kg/m³ yeast extract paste (Gist Brocades, The Netherlands), and 10 kg/m³ CaCO₃. At regular intervals the spores were subcultured with heat-shock treatment in fresh media. The number of tracings of the spores used for the batch experiments varied between 1 and 42 times.

Chemicals and media. The fermentation media consisted of various concentrations of carbohydrates (see Experimental results). Dried whey ultrafiltrate, which contained about 80% lactose, was obtained from the Laboratory of Dairy Research (NIZO, The Netherlands). Lactone monohydrate and galactose were obtained from Merck, Darmstadt, FRG. Unless stated otherwise all media contained yeast extract paste. The medium components were dissolved in distilled water and sterilized separately for 20–30 min at 110°C. The solution was at room temperature. Oxygen-free nitrogen was obtained by leading the gas through a heated cylinder of copper-tubings (300°C).

Batch experiments (1-1 scale). A 1.5 L Bioaldehyde fermentor was used for these experiments. A heat-shocked spore suspension was used as inoculum; the inoculation rate was 1:10. Flushing the gas-phase with nitrogen kept the system anaerobic. When necessary pH was adjusted with 2N NaOH at the beginning of each experiment. Samples were taken automatically at regular time intervals (every 4 h) by a fraction collector (Carlo-Erba), controlled by an OMRON SCY-PO sequence controller.

Batch experiments (200-ml scale). These experiments were carried out in flasks in a thermostatted water-bath. Each flask was stirred by a magnetic stirrer and kept anaerobic by flushing the gas-phase with nitrogen. The inoculation rate was 1:30 (heat-shocked spore suspension). No pH adjustment was applied. When all fermentation activity had ceased pH and end-product composition of the flasks were determined (120–150 h).

Assay methods. Lactose and galactose concentrations were determined by HPLC (Waters) on an HPX-87C column (Bio-Rad, 30 cm × 7.8 mm). Glucose concentrations were determined enzymatically (biotin kinase, Boehringer) or by HPLC. Butanol, isopropanol, butyric acid, and acetic acid concentrations were measured on a Packard-Becker gas chromatograph, type 437 (Chromosorb 106 column, 100–120 mesh; 140°C, 10°C/min, final hold 5 min, 170°C). The biomass concentrations in the liquid phase were determined as dry weight concentrations (dried 24 h at 110°C).

Experimental results and discussion
Batch experiments on 1-1 scale
For these experiments several combinations of carbohydrate and yeast extract paste were used. Table 1 lists these combinations.

Table 1. Fermentation media used on 1-1 scale

<table>
<thead>
<tr>
<th>Medium no</th>
<th>Composition</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>Whey ultrafiltrate (25 kg/m³), v.e. paste (13 kg/m³)</td>
</tr>
<tr>
<td>II</td>
<td>Whey ultrafiltrate (25 kg/m³), v.e. paste (6.5 kg/m³)</td>
</tr>
<tr>
<td>III</td>
<td>Whey ultrafiltrate (15 kg/m³), v.e. paste (13 kg/m³)</td>
</tr>
<tr>
<td>IV</td>
<td>Whey ultrafiltrate (50 kg/m³), v.e. paste (6.5 kg/m³)</td>
</tr>
<tr>
<td>V</td>
<td>Glucose (40 kg/m³), v.e. paste (13 kg/m³)</td>
</tr>
<tr>
<td>VI</td>
<td>Lactose, H₂O₂ (42 kg/m³), v.e. paste (13 kg/m³)</td>
</tr>
</tbody>
</table>

Table 2. Batch experiment on 1-4 scale

<table>
<thead>
<tr>
<th>Exp. Medium</th>
<th>T (°C)</th>
<th>pH</th>
<th>t (h)</th>
<th>Inoc.</th>
<th>JS (mg/m³)</th>
<th>JP (mg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Butanol</td>
<td>Isopropanol</td>
</tr>
<tr>
<td>I</td>
<td>37</td>
<td>5.1</td>
<td>80</td>
<td>23</td>
<td>6.9</td>
<td>0.4</td>
</tr>
<tr>
<td>II</td>
<td>37</td>
<td>5.0</td>
<td>140</td>
<td>1</td>
<td>7.4</td>
<td>0.0</td>
</tr>
<tr>
<td>III</td>
<td>37</td>
<td>5.2</td>
<td>80</td>
<td>1</td>
<td>6.2</td>
<td>0.9</td>
</tr>
<tr>
<td>IV</td>
<td>37</td>
<td>5.0</td>
<td>80</td>
<td>2</td>
<td>6.7</td>
<td>0.7</td>
</tr>
<tr>
<td>V</td>
<td>37</td>
<td>6.0</td>
<td>63</td>
<td>2</td>
<td>11.5</td>
<td>1.1</td>
</tr>
<tr>
<td>VI</td>
<td>37</td>
<td>6.5</td>
<td>93</td>
<td>2</td>
<td>13.3</td>
<td>0.7</td>
</tr>
<tr>
<td>VII</td>
<td>37</td>
<td>6.0</td>
<td>93</td>
<td>3</td>
<td>23.4</td>
<td>0.6</td>
</tr>
<tr>
<td>VIII</td>
<td>30</td>
<td>6.0</td>
<td>70</td>
<td>41</td>
<td>19.5</td>
<td>3.2</td>
</tr>
<tr>
<td>IX</td>
<td>30</td>
<td>6.5</td>
<td>150</td>
<td>41</td>
<td>24.0</td>
<td>5.0</td>
</tr>
<tr>
<td>X</td>
<td>30</td>
<td>5.2</td>
<td>100</td>
<td>42</td>
<td>17.0</td>
<td>4.0</td>
</tr>
<tr>
<td>XI</td>
<td>37</td>
<td>5.0</td>
<td>75</td>
<td>42</td>
<td>12.6</td>
<td>1.5</td>
</tr>
<tr>
<td>XII</td>
<td>37</td>
<td>6.0</td>
<td>47</td>
<td>37</td>
<td>29.8</td>
<td>5.4</td>
</tr>
<tr>
<td>XIII</td>
<td>30</td>
<td>5.4</td>
<td>100</td>
<td>42</td>
<td>37.9</td>
<td>7.7</td>
</tr>
<tr>
<td>XIV</td>
<td>30</td>
<td>5.4</td>
<td>80</td>
<td>42</td>
<td>39.4</td>
<td>7.7</td>
</tr>
</tbody>
</table>

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The experimental results are listed in Table 2. This Table gives for each experiment: the number of the medium used; the fermentation temperature, the pH at the beginning of the experiment, the fermentation time including lag-phase (t), the number of heat-shocked transfers the inoculum had undergone (Inoc), the concentration difference in carbohydrate due to fermentation (JS in kg/m³) and the concentrations of products formed (JP in kg/m³); t, JS and JP were determined by examination of the time-concentration course of each experiment.

The dry weight concentrations of biomass varied between ± 1.3 kg/m³ (expts. 1–5, 11 and 12) and ± 3.2 kg/m³ (expts. 8–10, 13, 14). Examination of Table 2 reveals that the concentration of butanol produced from whey ultrafiltrate is greatly increased by lowering the temperature from 37°C to 30°C, at 30°C the total amount of carbohydrate (lactose)
fermented is higher too. Variations of pH, yeast extract concentration and inoculum pretreatment do not lead to butanol production at 37° C. Compared to glucose fermentation the amount of butanol produced from whey fermentation is lower. In Table 3 the overall productivity, Y\textsubscript{pb}, and the mean reactor productivity, Prod, are compared for glucose and whey ultrafiltrate.

The overall product yields for glucose and lactose (from whey) do not differ very much. Mean reactor productivity is two to three times lower for lactose (from whey) than for glucose. From the concentration-time course of the experiments it appears that the lag-phase for whey fermentation is longer and the substrate conversion rate lower.

**Batch experiments on 200-ml scale**

In Table 4 the media used for these experiments are listed:

<table>
<thead>
<tr>
<th>Medium no.</th>
<th>Composition*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Whey ultrafiltrate (50 kg/m\textsuperscript{3})</td>
</tr>
<tr>
<td>II</td>
<td>Whey ultrafiltrate (50 kg/m\textsuperscript{3})</td>
</tr>
<tr>
<td>III</td>
<td>Whey ultrafiltrate (100 kg/m\textsuperscript{3})</td>
</tr>
<tr>
<td>IV</td>
<td>Whey ultrafiltrate (150 kg/m\textsuperscript{3})</td>
</tr>
<tr>
<td>V</td>
<td>Lactose, H\textsubscript{2}O (100 kg/m\textsuperscript{3})</td>
</tr>
<tr>
<td>VI</td>
<td>Glucose (40 kg/m\textsuperscript{3})</td>
</tr>
<tr>
<td>VII</td>
<td>Galactose (40 kg/m\textsuperscript{3})</td>
</tr>
<tr>
<td>VIII</td>
<td>Glucose (10 kg/m\textsuperscript{3}) + Galactose (10 kg/m\textsuperscript{3})</td>
</tr>
<tr>
<td>IX</td>
<td>Glucose (20 kg/m\textsuperscript{3}) + Galactose (20 kg/m\textsuperscript{3})</td>
</tr>
</tbody>
</table>

* Mean reactor productivity was calculated from the concentration-time course of each experiment (lag-phase included).

Table 5. Batch experiments on 200-ml scale. All concentrations are final concentrations: JS - L3

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Medium</th>
<th>Lactose (kg/m\textsuperscript{3})</th>
<th>Glucose (kg/m\textsuperscript{3})</th>
<th>Galactose (kg/m\textsuperscript{3})</th>
<th>δS (kg/m\textsuperscript{3})</th>
<th>Butanol (kg/m\textsuperscript{3})</th>
<th>Isopropanol (kg/m\textsuperscript{3})</th>
<th>Butyrate (kg/m\textsuperscript{3})</th>
<th>Acetate (kg/m\textsuperscript{3})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>10.7</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>3.7</td>
<td>0.4</td>
<td>1.4</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>I</td>
<td>11.6</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>3.0</td>
<td>0.2</td>
<td>2.3</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>I</td>
<td>14.1</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>2.1</td>
<td>0.2</td>
<td>2.1</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>II</td>
<td>18.9</td>
<td>-</td>
<td>-</td>
<td>21</td>
<td>5.2</td>
<td>1.4</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>5</td>
<td>II</td>
<td>19.1</td>
<td>-</td>
<td>-</td>
<td>21</td>
<td>4.5</td>
<td>1.1</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>6</td>
<td>III</td>
<td>55.1</td>
<td>-</td>
<td>-</td>
<td>25</td>
<td>5.8</td>
<td>2.0</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>7</td>
<td>III</td>
<td>62.4</td>
<td>-</td>
<td>-</td>
<td>17</td>
<td>5.1</td>
<td>1.8</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>8</td>
<td>III</td>
<td>54.2</td>
<td>-</td>
<td>-</td>
<td>26</td>
<td>5.8</td>
<td>2.0</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>9</td>
<td>IV</td>
<td>91.9</td>
<td>-</td>
<td>-</td>
<td>28</td>
<td>4.9</td>
<td>1.7</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td>V</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>8.0</td>
<td>2.0</td>
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<tr>
<td>11</td>
<td>V</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>7.1</td>
<td>2.2</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>12</td>
<td>VI</td>
<td>2.3</td>
<td>-</td>
<td>-</td>
<td>38</td>
<td>3.8</td>
<td>3.5</td>
<td>0.0</td>
<td>0.7</td>
</tr>
<tr>
<td>13</td>
<td>VI</td>
<td>6.2</td>
<td>-</td>
<td>-</td>
<td>34</td>
<td>7.0</td>
<td>4.3</td>
<td>0.0</td>
<td>0.6</td>
</tr>
<tr>
<td>14</td>
<td>VII</td>
<td>-</td>
<td>21.6</td>
<td>-</td>
<td>18</td>
<td>3.8</td>
<td>1.2</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>15</td>
<td>VII</td>
<td>-</td>
<td>24.5</td>
<td>-</td>
<td>16</td>
<td>3.6</td>
<td>1.0</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>16</td>
<td>VIII</td>
<td>0.0</td>
<td>0.0</td>
<td>0.5</td>
<td>19</td>
<td>3.4</td>
<td>1.6</td>
<td>1.4</td>
<td>0.2</td>
</tr>
<tr>
<td>17</td>
<td>VIII</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>20</td>
<td>3.2</td>
<td>1.6</td>
<td>1.5</td>
<td>0.2</td>
</tr>
<tr>
<td>18</td>
<td>IX</td>
<td>0.0</td>
<td>0.0</td>
<td>11.1</td>
<td>29</td>
<td>5.4</td>
<td>3.0</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>19</td>
<td>IX</td>
<td>0.0</td>
<td>14.3</td>
<td>-</td>
<td>26</td>
<td>3.5</td>
<td>2.1</td>
<td>1.5</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Biomass dry weight concentrations varied between 1.5 and 3.0 kg/m\textsuperscript{3}. The results from the 200-ml experiments again confirm what was found on the 1-1 scale: Cl. bverinckii LMD 27.6 is capable of producing butanol from whey ultrafiltrate at 30° C. Furthermore, it seems that higher whey concentrations lead to a higher lactose conversion (expts. 1-9). Glucose/galactose mixtures also yield butanol at 30° C; a preference for glucose is observed.

**Conclusions**

Butanol can be produced from whey ultrafiltrate by Cl. bverinckii LMD 27.6 in batch cultures at 30° C. Solvent productivity for whey fermentations is two to three times lower than productivity found in glucose fermentations. Mixtures of glucose and galactose can also be fermented to butanol, although a preference for glucose as a substrate is found.

With the data from the batch experiments a program is set up to find ways of producing butanol continuously from whey ultrafiltrate with immobilized Cl. bverinckii cells. Both direct and indirect fermentation of whey ultrafiltrate are considered.

**Acknowledgement.** We wish to thank the Dutch Institute of Dairy Research (NZO) for financial support.

**References**


Received November 4, 1983
Continuous butanol production from whey permeate with immobilized Clostridium beyerinckii LMD 27.6.

Continuous butanol production from whey permeate with immobilized *Clostridium beyerinckii* LMD 27.6

G. H. Schoutens, M. C. H. Nieuwenhuizen, and N. W. F. Kossen

Laboratory of Bioengineering, Department of Chemical Engineering, Delft University of Technology

Summary. The aim of this study was to find the conditions necessary for the continuous butanol production from whey permeate with *Clostridium beyerinckii* LMD 27.6, immobilized in calcium alginate beads. The influence of three parameters on the butanol production was investigated: the fermentation temperature, the dilution rate (during start-up and at steady state) and the concentration of calcium ions in the fermentation broth. It was found that both a fermentation temperature of 30°C and a dilution rate of 0.1 h⁻¹ or less during the start-up phase are required to achieve continuous butanol production from whey permeate. Butanol can be produced continuously from whey permeate in reactor productivity six times higher than those found in batch cultures with free *C. beyerinckii* cells on whey media.

Introduction

During the last decade the interest in the fermentative production of alcohols in continuous processes with immobilized cells has grown steadily (Cheetham 1980, Chihara et al. 1983, Fukui et al. 1982). Under favourable conditions high reactor productivities and high biomass concentrations can be achieved without wash-out of the cells.

Krouwel described the continuous production of butanol from glucose with *Clostridium beyerinckii* LMD 27.6, immobilized in calcium alginate beads (Krouwel et al. 1983a). For a possible large scale application several aspects of this process have to be optimized, one of which is the substrate used.

Whey permeate is a by-product of cheese manufacture, containing mainly lactose and salts; in some countries this substrate could offer more perspective for the butanol fermentation than glucose (Lenz et al. 1980). Various strains of *C. acetobutylicum* were used for batch fermentations of whey (Welch et al. 1984, Maddox 1980, Gapes et al. 1982), but little is known about the lactose fermenting ability of *C. beyerinckii* LMD 27.6.

Batch experiments with free cells and continuous experiments with immobilized cells on whey media were set up. The results of these experiments were hard to use directly for the continuous experiments: both continuous processing (Jobbes et al. 1983) and immobilization can seriously affect the metabolism of the cells. As the first attempts to achieve continuous butanol production from whey permeate with immobilized cells failed, three parameters were thought to influence the fermentation: 1. the fermentation temperature; 2. the dilution rate; and 3. the calcium chloride concentration.

Calcium ions are used for the stability of the alginate beads (Klein et al. 1979). *Krouwel* did not find an inhibitory effect of calcium ions (5 kg/m³ CaCl₂; 2H₂O) in glucose fermentations with free *C. beyerinckii* cells (Krouwel et al. 1983a). However, indications exist that lactose hydrolysing enzymes like β-galactosidase can be inhibited by calcium ions (Brooks 1983). This is not known which enzymes in *C. beyerinckii* are used for the lactose utilization and whether they are inhibited by calcium ions, therefore the influence of omission of calcium chloride from the medium on the fermentation was considered.

Materials and methods

*Micro-organisms.* *Clostridium beyerinckii* LMD 27.6 was obtained from the Laboratory of Microbiology, Delft University of Technology. It was subcultured (with heat shock treatment) seven times at 37°C on a medium of 60 kg/m³ glucose (Baker, The Netherlands). 13 kg/m³ yeast extract paste and 10 kg/m³ CaCO₃ and kept at a spore suspension.

*Chemicals and media.* Dried whey permeate (whey 85% lactose, was obtained from the Dutch Institute of Dairy Research (NIZO), The Netherlands).

*Fermentation media.* The fermentation media for the continuous cultures contained glucose (20 kg/m³) or whey permeate (5 kg/m³, 50 kg/m³ yeast extract paste and 10 kg/m³ CaCl₂) and kept as a spore suspension.

**Chemicals and media.** Dried whey permeate (whey 85% lactose) containing about 80% lactose, was obtained from the Dutch Institute of Dairy Research (NIZO), The Netherlands.

The fermentation media for the continuous cultures contained glucose (20 kg/m³) or whey permeate (5 kg/m³, 50 kg/m³ yeast extract, 10 kg/m³, Difco) and CaCl₂·2H₂O (0 or 5 kg/m³), dissolved in distilled or demineralized water. All medium components were sterilized separately at 110°C and mixed at room temperature. Sodium alginate (Manzou LD) was obtained via ICIL, Rotterdam from Alginic Ind. (Great Britain). Oxygen-free nitrogen was obtained by leading the gas through a column with copper shavings (50°C).

**Assay methods.** The same methods and conditions as described by Schoutens (Schoutens et al. 1984) were used. In samples of whey experiments lactose, glucose, and galactose concentrations were determined, the glucose and galactose concentrations were always below detection level.

**Immobilization.** Per liter distilled water 60 g wet spore paste and 80 g sodium alginate were dissolved; the spore paste was harvested from a 15 L batch fermentation on glucose or whey permeate. The suspension containing spores and alginate was pumped through hypodermic needles with a concentric air flow into a bath of 30 kg/m³ CaCl₂·2H₂O in distilled water, where the calcium alginate beads were formed. The beads were kept in this solution for 3—5 h transferred to a 5 kg/m³ CaCl₂·2H₂O solution and stored at 4°C (bead diameter about 2.0 ± 0.1 mm).

**The continuous fermentations.** The experimental set-up described by *Krouwel* was used (Krouwel et al. 1983b). The fermenter (a stirred tank, working volume 400 or 600 ml) was sterilized (115°C, 30 min) and filled with alginate beads. Then the fermenter was filled with a 50% ethanol/water mixture and stirred for 30 min to deactivate any contaminants introduced during the nonaseptic immobilization procedure. The reactor was washed three times with a sterile CaCl₂·2H₂O solution (5 kg/m³) and three times with sterile fermentation medium.

**The feed was continuously injected through a septum at the top of the fermenter; medium outflow was controlled by a level control system (using a conductivity measurement). The fermentation media for the continuous cultures contained: a) glucose (20 kg/m³), 5 kg/m³ CaCl₂·2H₂O; 10 kg/m³ yeast extract; 190–225 kg/m³ whey permeate (20 kg/m³ lactose), 5 kg/m³ CaCl₂·2H₂O, 10 kg/m³ yeast extract; b) product concentration, Cₚ (kg/m³) switched at t = 190 h from glucose to whey permeate with all other conditions remaining the same.

**1. Influence of the fermentation temperature**

Two experiments showed that the fermentation temperature was of great influence. In Fig. 2 the first of these experiments is shown: a butanol producing culture at 37°C is switched from glucose to whey permeate, the fermentation temperature was lowered...
From these experiments it is clear that 30°C is essential for continuous butanol production from whey permeate. Furthermore neither the whey permeate concentration (25 or 50 kg/m³) nor the substrate used during start-up (glucose or whey permeate) influence the fermentation pattern.

2. Influence of the dilution rate

During the start-up phase. In the usual start-up procedure of a continuous culture the dilution rate is kept at 0.1 h⁻¹ or less during the first 24–48 h, which spurn germination in the beads takes place (see Materials and methods). Twice it was attempted to start a continuous culture on whey media (25 kg/m³ whey permeate, 10 kg/m³ yeast extract and 5 kg/m³ CaCl₂; 2 H₂O, 30°C) at dilution rates higher than 0.1 h⁻¹: 0.30 and 0.25 h⁻¹ respectively. The results were dramatic: hardly any fermentation activity was observed at all, no steady states were established and the lactose conversion was 1–3 kg/m³ at the most. A dilution rate of 0.1 h⁻¹ or less during the start-up phase seems essential for germination of the spores in the alginate beads, although it is not at all clear what is the reason for this phenomenon. Preliminary results indicate that this phenomenon is also present in glucose fermentations.

During steady state. In two experiments at 30°C on whey media, started on a low dilution rate, steady state butanol productions were measured at different dilution rates (all other parameters were kept constant during these experiments). In Fig. 3 the reactor productivities [in kg (butanol + isopropanol)/m³ h⁻¹] are presented as a function of the dilution rate. At a dilution rate of 0.1 h⁻¹ the system seems to produce butanol and isopropanol at its maximal rate; the same tendency was found for continuous glucose fermentations (Krouwel et al. 1983b).

3. Influence of the calcium chloride concentration

No (positive or negative) influence of calcium ions (in 5 kg/m³ CaCl₂; 2 H₂O) on the continuous butanol production was found (as was already clear from the temperature effect found in section 1. Media without calcium ions led to the expected disintegration of the alginate beads.

In Table 1 the reactor productivities of the continuous experiments with immobilized cells are presented next to those of similar experiments on glucose media at 37°C and to those of batch experiments with free cells. All experiments were carried out in stirred tank reactors, all media to 30°C and the calcium chloride was omitted from the medium.

The result was a steady butanol production (2.1 kg/m³ butanol) and a lactose conversion of 7.2 kg/m³.

The second experiment was a continuous culture at 30°C, started on a medium of 25 kg/m³ whey permeate, 10 kg/m³ yeast extract and 5 kg/m³ CaCl₂; 2 H₂O and continuously producing butanol; increase of the fermentation temperature to 37°C (with all other conditions constant) led to a rapid decrease of the butanol concentration (from 2.6 to 0 kg/m³) and an increase of the butyrate concentration (from 0.2 to 1.2 kg/m³).

References


G. H. Schoutens et al.: Butanol production from whey permeate with Clostridium beyerinckii LMD 27.6

Table 1. Reactor productivities from experiments with C. beyerinckii LMD 27.6 [kg (butanol + isopropanol)/m³ h⁻¹]

<table>
<thead>
<tr>
<th>Batch experiments</th>
<th>Continuous experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose media at 37°C</td>
<td>0.220 ± 0.04</td>
</tr>
<tr>
<td>Whey media at 30°C</td>
<td>0.050 ± 0.05</td>
</tr>
</tbody>
</table>

* Krouwel et al. 1983a
Schoutens et al. 1984
Krouwel et al. 1983b

For batch experiments the productivities were calculated from concentration butanol + isopropanol/Fermentation time (iod phase).

From the continuous experiments with Clostridium beyerinckii LMD 27.6 it can be concluded that:

- both a fermentation temperature of 30°C and a dilution rate of 0.1 h⁻¹ or less during the start-up phase of the fermentation are required to achieve continuous butanol production from whey permeate;
- butanol can be produced continuously from whey permeate in reactor productivities sixteen times higher than those found in batch cultures with free C. beyerinckii cells on whey media.

Acknowledgements. We wish to thank Dr. I. S. Maddox, Dr. J. D. Brooks and their co-workers for their interest and ideas concerning our project and the Dutch Institute of Dairy Research (NIZO) for financial support.

Conclusions

From the continuous experiments with Clostridium beyerinckii LMD 27.6 it can be concluded that:

- both a fermentation temperature of 30°C and a dilution rate of 0.1 h⁻¹ or less during the start-up phase of the fermentation are required to achieve continuous butanol production from whey permeate;
- butanol can be produced continuously from whey permeate in reactor productivities sixteen times higher than those found in batch cultures with free C. beyerinckii cells on whey media. Further optimization and an economic evaluation are needed to see if the process is suitable for a large scale application.
G. H. Schoutens et al.: Butanol production from whey permeate with Clostridium beyerinckii LMD 27.6


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A simple model for the continuous production of butanol by immobilized Clostridia.

Part I: Cl. beyerinckii on glucose.

A SIMPLE MODEL FOR THE CONTINUOUS PRODUCTION OF BUTANOL BY IMMOLIZED CLOSTRIDIA.

Part I: Cl.beyerinckii on glucose

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SUMMARY

A simple model was developed to describe the continuous production of butanol and isopropanol from glucose by Clostridium beyerinckii cells, immobilized in calcium alginate beads. The model contains linear butanol inhibition kinetics and assumes the biomass to be homogeneously distributed over the alginate beads. For design purposes this model, containing five parameters, satisfactorily predicts the values of three output variables (the concentrations of glucose, butanol and isopropanol) as a function of three input variables (the glucose feed concentration, the dilution rate and the fraction of beads in the reactor). For process design a high beadsfraction and well mixed conditions seem advantageous.

INTRODUCTION

As immobilized cell systems become more widely used, kinetic models to describe the performance of such systems will be needed.

In the field of waste water treatment relatively simple, unstructured models are found to satisfactorily describe the substrate consumption in biofilm reactors\(^1\). The aim of this study was to find a simple model to describe and predict the continuous production of butanol and isopropanol by Clostridium beyerinckii cells, immobilized in calcium alginate beads. Kinetic models describing the batch and continuous production of alcohols using immobilized cells have been published\(^3\). However, when complex systems with producing and growing immobilized cells are considered, steady state values are hard to obtain and often show relatively large variations. This of course makes parameter estimation difficult and discrimination between models hard; models with as little parameters as possible are therefore desired. A model containing seven parameters was developed\(^5\) to describe the production of and inhibition by ethanol in a gas lift reactor with yeast flocs. This straightforward model gave satisfactory results and could be used for design purposes.

For the continuous butanol/isopropanol production by immobilized Clostridia a 9 parameter model was developed by Krouwel\(^4\). However, this model did not account for product inhibition. Furthermore, the immobilization matrix was modelled as an inhomogeneous sphere with an inactive core. As new information about product inhibition kinetics and about the biomass distribution in the immobilization matrix was found, an attempt was made to develop a simplified model for this process, which could be used for process design.

THEORY

For the substrate consumption rate of the Clostridia the Herbert/Pirt equation\(^6\) is used:
$$r_s = \frac{1}{s_x}r_x + m_s c_x$$

(1)

with $r_s =$ substrate consumption rate (kg/m$^3$ hr)
$r_x =$ biomass production rate (kg D.W./m$^3$ hr)
$Y_{sx} =$ biomass yield factor (kg D.W./kg)
$m_s =$ maintenance factor (kg/kg D.W. hr)
$c_x =$ biomass concentration (kg D.W./m$^3$)

The biomass production rate is written as:

$$r_x = \mu c_x$$

(2)

with $\mu =$ specific growth rate (hr$^{-1}$)

The specific growth rate is expressed as:

$$\mu = \mu_{max} f_1(c_s) f_2(c_p)$$

(3)

with $\mu_{max} =$ maximum specific growth rate (hr$^{-1}$)

In general for these processes Monod kinetics is used to describe the substrate concentration dependency of the specific growth rate:

$$f_1(c_s) = \frac{c_s}{c_s + K_s}$$

(4)

with $c_s =$ substrate concentration (kg/m$^3$)
$K_s =$ Monod constant (kg/m$^3$)

The inhibition of product on the specific growth rate is expressed by $f_2(c_p)$

For ethanol producing systems various unstructured models to describe the inhibition kinetics have been proposed$^4,6$. These models can often be described by equations of the form:

$$f_2(c_p) = (1 - \frac{c_p}{c_{p,max}})^n$$

(5)

with $c_p =$ concentration inhibiting product (kg/m$^3$)
$c_{p,max} =$ maximum conc. inhibiting product (kg/m$^3$)
$n =$ polynomial degree

When equation 5 is used to describe the product inhibition in the butanol/isopropanol fermentation and Monod kinetics is used, equation 1 gives:

$$r_s = \frac{\mu_{max} c_x}{Y_{sx} (c_s + K_s)} (1 - \frac{c_p}{c_{p,max}})^n + m_s c_x$$

(6)

To describe the substrate consumption and product formation by immobilized Clostridia in a well mixed reactor a non-gradient model is used; external diffusion limitations are considered to be absent. Furthermore the biomass is considered to be homogeneously distributed throughout the alginate beads.

The substrate balance over the reactor at steady state is given by:

$$\phi_v c_{SO} = \phi_v c_S + V_t n(1-c) r_s$$

(7)

with $\phi_v =$ feed flow rate (m$^3$/hr)
$c_{SO} =$ feed substrate concentration (kg/m$^3$)
$c_S =$ substrate concentration (kg/m$^3$)
The substrate consumption rate is calculated from equation 6. The biomass in the reactor consists of two parts: the immobilized cells (with a concentration $C_{xs}$ (kg D.W./m$^3$ alginate)) and the freely suspended cells (with a concentration $C_{xl}$ (kg D.W./m$^3$)). As an approximation, $C_{xl}$ is considered to be constant at steady state. It is assumed that newly formed cells (due to growth) wash out of the immobilization matrix into the fermentation broth. Furthermore, it is assumed that these freely suspended cells have a negligible effect on the total substrate consumption rate. Therefore, to calculate $r_s$ from equation 6, the biomass concentration $C_x$ is substituted by the immobilized biomass concentration $C_{xs}$. This approximation is implicitly used in equations 7 and 8; $r_s$ used in these equations is expressed as (kg/m$^3$ alginate hr).

The effectiveness factor $\eta$ is defined as the ratio between the actual conversion rate and the conversion rate that would occur if no diffusional limitation in the carrier material was present. For spherical geometries this is:

$$\eta = \frac{4 \pi R^2 \rho \left(\frac{dC}{dr}\right)}{3 \pi R^3 r_s} \bigg|_{r=R}$$

(9)

The effectiveness factor can be derived from e.g. the generalized modulus $\phi$:

$$\phi = \frac{n+1}{2} \frac{r_s}{D_e C_s}$$

(10)

with $n$ = order of reaction.

In steady state the product concentrations (butanol and isopropanol) can be calculated from the amount of substrate consumed,

$$C_B = Y_{sb} (C_{s0} - C_s)$$

(11)

with $C_B$ = butanol concentration (kg/m$^3$)

$Y_{sb}$ = butanol yield factor (kg/kg)

or via the reduction degree balance.

Furthermore, butanol and isopropanol are assumed to be formed in a constant ratio $k$:

$$C_{ip} = \frac{C_B}{k}$$

(12)

with $C_{ip}$ = isopropanol concentration (kg/m$^3$)

$k$ = ratio butanol/isopropanol.

Rearrangement and substitution of equations 6, 8, 11 and 12; substitution of $Y_{sx}$ by $r_{max}$ leads to the following model:
When the three input variables \( D_t, (1-e) \) and \( C_{S0} \) are known an iteration procedure (calculating \( n \) for every \( C_S \)) leads to the three output variables \( (C_s, C_B \) and \( C_{IP}) \).

**MATERIALS AND METHODS**

**Micro-organism:** The anaerobic spore-former *Clostridium beyerinckii* LMD 27.6 was obtained from the Laboratory of Microbiology, Delft. The organism was cultured and immobilized as described earlier.

**Experimental conditions:** The fermentation media contained glucose (20-60 kg/m³), yeast extract (Difco, 10 kg/m³) and calcium chloride (Merck, 5 kg/m³). The medium components were sterilized separately at 110 °C and mixed at room temperature.

The experiments were carried out in well mixed reactors: a CSTR (1 l. working volume) and a glass column reactor (0.2 l. working volume). In the latter one liquid mixing was obtained by high recirculation rates of the fermentation broth. The start up procedure of the reactors was described earlier. The fermentation temperature was 37 °C.

**Assay methods:** glucose concentrations were determined enzymatically. The concentrations of butanol and isopropanol (main products) and butyrate and acetate (by products) were measured gaschromatographically. The alginate fraction in the fermenter \((1-e)\) was determined by measuring total and liquid volumes. The biomass concentrations in the liquid phase were determined as dry weight concentrations. The biomass concentrations in the alginate beads were determined by dissolving the beads in a 0.2 N sodiumphosphate/0.2 N sodiumcitrate solution containing 15 drops of 4% formaldehyde (at pH 6.3). Stirring, centrifugation and drying leads to the dry weight concentration of biomass. Substraction of the dry weight fractions of used and unused beads leads to the dry weight contents of alginate beads. The biomass distribution was determined by electronmicrographing alginate beads, fixed in resins (carried out by the Lab. of Microbiology, Delft). The fixed beads were sliced with a microtome and slabs out of the center were used for photographs. The computer calculations were performed on a HP1000/A900 computer (Hewlett Packard).

**EXPERIMENTAL RESULTS AND DISCUSSION**

**Continuous experiments:** In twelve continuous experiments the steady state glucose and product concentrations were determined for various values of \( C_{S0}, \) \( D_t \) and \( (1-e) \). In table 1 the results of these experiments are listed (the steady state values were calculated from intervals in which the output variables varied 10% or less during a period of at least eight liquid residence times). Small amounts of acetic acid and butyric acid (< 0.4 kg/m³) were formed with the main products butanol and isopropanol. Furthermore, the dry weight concentrations of biomass in the fermentation broth were about 1.5 kg/m³ or less.
Table 1: Results of the continuous experiments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$D_{t}$ (hr$^{-1}$)</th>
<th>$(1-c)$ (%)</th>
<th>$C_{SO}$ (kg/m$^3$)</th>
<th>$C_{S}$ (kg/m$^3$)</th>
<th>$C_{B}$ (kg/m$^3$)</th>
<th>$C_{IP}$ (kg/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.11</td>
<td>15.0</td>
<td>55.0</td>
<td>35.4</td>
<td>4.3</td>
<td>2.3</td>
</tr>
<tr>
<td>2</td>
<td>0.16</td>
<td>20.0</td>
<td>58.5</td>
<td>40.2</td>
<td>3.9</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>0.16</td>
<td>30.0</td>
<td>20.0</td>
<td>1.5</td>
<td>3.5</td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
<td>0.23</td>
<td>30.0</td>
<td>19.8</td>
<td>2.0</td>
<td>4.2</td>
<td>2.3</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td>30.0</td>
<td>30.0</td>
<td>13.3</td>
<td>3.9</td>
<td>2.5</td>
</tr>
<tr>
<td>6</td>
<td>0.26</td>
<td>50.0</td>
<td>30.0</td>
<td>9.9</td>
<td>4.1</td>
<td>2.5</td>
</tr>
<tr>
<td>7</td>
<td>0.28</td>
<td>30.0</td>
<td>27.8</td>
<td>14.2</td>
<td>3.7</td>
<td>1.7</td>
</tr>
<tr>
<td>8</td>
<td>0.28</td>
<td>40.0</td>
<td>60.0</td>
<td>41.0</td>
<td>3.4</td>
<td>1.8</td>
</tr>
<tr>
<td>9</td>
<td>0.35</td>
<td>30.0</td>
<td>20.0</td>
<td>4.5</td>
<td>3.4</td>
<td>2.0</td>
</tr>
<tr>
<td>10</td>
<td>0.42</td>
<td>50.0</td>
<td>29.7</td>
<td>12.8</td>
<td>3.5</td>
<td>2.0</td>
</tr>
<tr>
<td>11</td>
<td>0.48</td>
<td>37.0</td>
<td>29.6</td>
<td>12.6</td>
<td>4.5</td>
<td>2.3</td>
</tr>
<tr>
<td>12</td>
<td>0.88</td>
<td>50.0</td>
<td>30.3</td>
<td>17.7</td>
<td>2.4</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Inhibition kinetics: The form of the product inhibition curve for this fermentation was tested in another series of continuous experiments with immobilized cells; free Cl. beyerinokii cells do not produce substantial amounts of butanol and isopropanol under these conditions. By addition of butanol (in the range of 2.5–5.0 kg/m$^3$) it was found that the substrate consumption rate (kg/m$^3$ alginate hr) decreased linearly. In figure 1 the course of such a butanol addition experiment is shown. For batch experiments with Cl. acetobutylicum the same phenomenon was described in the literature and linear butanol inhibition kinetics was chosen to describe this.

The butanol addition experiments led to the conclusion that equation 5 (theory) for this process can be simplified to:

$$f_2(C_B) = \left( 1 - \frac{C_B}{C_{B,max}} \right)$$  \hspace{1cm} (5a)

and from these experiments $C_{B,max}$ was estimated to be 5.5 kg/m$^3$.

Figure 1: Typical course of the butanol inhibition curve.
Modelling

With equations 13 and 5a three models were constructed to describe the substrate consumption and alcohol production by the immobilized cells. Scheme 1 depicts the equations of which each model exists. Models 2 and 3 are simplifications of model 1.

Scheme 1: Arrangement of models 1, 2 and 3

- Model 1 assumes Monod kinetics, butanol inhibition and maintenance energy requirements; model 2 uses zero order kinetics and butanol inhibition and model 3 consists of mere zero order kinetics to describe the substrate consumption.

Model parameters: The glucose diffusion coefficient, necessary to calculate \( \eta \), was taken from the literature \( D_e = 4.8 \times 10^{-10} \, \text{m}^2/\text{s} \). The ratio butanol/isopropanol (k) was estimated to be 2 kg/kg from literature data. The maximum butanol concentration \( (C_{B,max}) \) was estimated to be 5.5 kg/m^3, as stated in the previous section; The butanol yield, \( Y_{sb} \), was calculated from the twelve experiments in table 1: 0.22 ± 0.03 kg/kg. The remaining parameters are \( r_{max} \), \( K_s \) and \( M (= m_s C_{XS}) \). They were estimated from the twelve experiments (table 1) by a direct grid search parameter estimation method. This parameter estimation was based on one output variable: the glucose concentration \( C_g \).

For models 1, 2 and 3 the parameters were estimated; the accuracy of the fit between model and experiments (12 for all models) is expressed in the residual sum of squares \( \text{RSS} = \sum (C_{S,calculated} - C_{S,measured})^2 \). These RSS values can be compared directly with each other and with the experimental variance.

Model 1 contains seven parameters; the effectiveness factor, \( \eta \), was calculated to be 1. Furthermore, the Monod constant, \( K_s \), was estimated to be 0.4 kg/m^3; \( r_{max} \) was 40 kg/m^3 alginate hr and the maintenance factor \( M (= m_s C_{XS}) \) was 0.5 kg/m^3 alginate hr. The residual sum of squares (RSS) was found to be 18 for the twelve experiments.

Model 2 exists of five parameters; again the effectiveness factor was found to be 1 for all experiments. The one parameter that remained to be estimated was \( r_{max} \); a value of 50 kg/m^3 alginate hr was found. The RSS value for the twelve experiments was 20.

Model 3 uses four parameters; \( \eta \) was found to be unity again. For \( r_{max} \), a value of 13 kg/m^3 alginate hr was estimated and RSS was 165 for the 12 experiments.
Model discrimination and evaluation: The experimental variance in the twelve experiments can be estimated from the steady state fluctuations (~ 10%). The mean variance, \( \Sigma (\Delta C_{s,\text{obs.}})^2 \), for all twelve experiments would then be approximately 50. Discrimination between the models with a model discrimination computer program gave a rejection of model 3. Discrimination between models 1 and 2 was impossible, they fit experiments 1-12 equally well. Therefore model 2, with the least number of parameters was chosen to describe experiments 1-12.

The \( r_{\text{max}} \) value in model 2 was found to be 50 kg/m\(^3\) alginate hr. If we recall that \( r_{\text{max}} = \mu_{\text{max}} Y_{\text{S}} / X_{\text{S}} \) and that \( Y_{\text{S}} \) values for this system vary between 0.05-0.1 kg D.W./kg, then \( \mu_{\text{max}} \) would approximately be 0.06-0.13 hr\(^{-1}\). As no literature data for \( \mu_{\text{max}} \) of immobilized growing Clostridia cells were found, the calculated \( \mu_{\text{max}} \) value was compared with an experimentally determined one. Experiment 12 shows a large residual sugar concentration and a relatively low butanol concentration (low inhibition). In this experiment \( \mu \) would therefore approximate \( \mu_{\text{max}} \).

An estimation of \( \mu \) is given by: \( C_{\text{S}} \Delta t / (1-\epsilon) C_{X_S} \). For experiment 12 this leads to an estimated \( \mu \) of 0.07 hr\(^{-1}\). This means that the calculated \( \mu_{\text{max}} \) values are in the same range as those experimentally found.

In table 2 the observed and calculated (via model 2) output variables are given for experiments 1-12.

**Table 2:** Comparison of the observed and calculated (via model 2) values of the output variables for experiments 1-12.

<table>
<thead>
<tr>
<th>Exp.nr.</th>
<th>( C_{\text{S}} ) (kg/m(^3))</th>
<th>( C_{\text{B}} ) (kg/m(^3))</th>
<th>( C_{\text{IP}} ) (kg/m(^3))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>obs.</td>
<td>calc.</td>
<td>obs.</td>
</tr>
<tr>
<td>1</td>
<td>35.4</td>
<td>36.7</td>
<td>4.3</td>
</tr>
<tr>
<td>2</td>
<td>40.2</td>
<td>40.6</td>
<td>3.9</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>0.25</td>
<td>3.5</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>1.75</td>
<td>4.2</td>
</tr>
<tr>
<td>5</td>
<td>13.3</td>
<td>13.3</td>
<td>3.9</td>
</tr>
<tr>
<td>6</td>
<td>9.9</td>
<td>10.2</td>
<td>4.1</td>
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<td>41.0</td>
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<td>12.6</td>
<td>14.4</td>
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</tr>
<tr>
<td>12</td>
<td>17.7</td>
<td>17.0</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Consequences for process design: From the form of the product inhibition curve (figure 1) it can be concluded that for this system there is a slight preference for a reactor with a plug flow character, rather than one with a mixed flow character. But Krouwel described that a plug flow character during the start-up phase of the process does not provide the conditions optimal for spore germination. This and the fact that the escape of fermentation gas produced is facilitated in a well mixed reactor, leads to the conclusion that a well mixed reactor is to be preferred for the continuous production of butanol and isopropanol by immobilized Clostridia.
Also, (1-\(\varepsilon\)) is another important parameter for the process design: a well mixed reactor with high (1-\(\varepsilon\)) values will be optimal for this process. As was discussed earlier, model 2 fits the experiments 1-12 well, although the model is not very refined. Especially in the area where \(C^*\) values are low, the model will not accurately describe the substrate utilization (no Monod kinetics).

\[(1-\varepsilon) = 0.5\]

\[
\begin{array}{c|c|c|c|c|c|c}
\hline
\text{D} & 1 & 2 & 3 & 4 & 5 & 6 \\
\hline
\text{Prod (kg/m}^3\text{.hr)} & 0.5 & 0.6 & 0.7 & 0.8 & 0.9 & 1.0 \\
\hline
\end{array}
\]

\[(1-\varepsilon) = 0.3\]

\[
\begin{array}{c|c|c|c|c|c|c}
\hline
\text{D} & 1 & 2 & 3 & 4 & 5 & 6 \\
\hline
\text{Prod (kg/m}^3\text{.hr)} & 0.3 & 0.4 & 0.5 & 0.6 & 0.7 & 0.8 \\
\hline
\end{array}
\]

\[(1-\varepsilon) = 0.1\]

\[
\begin{array}{c|c|c|c|c|c|c}
\hline
\text{D} & 1 & 2 & 3 & 4 & 5 & 6 \\
\hline
\text{Prod (kg/m}^3\text{.hr)} & 0.1 & 0.2 & 0.3 & 0.4 & 0.5 & 0.6 \\
\hline
\end{array}
\]

Figure 3: The reactor productivity (kg butanol + isopropanol/m\(^3\).hr) calculated as a function of the dilution rate and reactor loading.

The effectiveness factors will still be larger than 80% at sugar concentrations of 1-2 kg/m\(^3\), but the discrepancy between model and experiments will be caused by the fact that zero-order substrate consumption is unlikely at low \(C_s\) values. However, for design purposes this model performs satisfactorily. The reactor design and scaling up aspects are the subject of a later study.

CONCLUSIONS

A simple, unstructured model with five parameters was developed to describe the continuous production of butanol and isopropanol from glucose by \textit{C. b.} cells, immobilized in calcium alginate beads. The model is based on linear butanol inhibition kinetics and assumes a homogeneous biomass distribution in the alginate beads. For design purposes this model satisfactorily predicts the value of three output variables (\(C_s^*, C_b^*, C_{IP}\)) as a function of three input variables (\(C_{SO}, D, 1-\varepsilon\)).

For optimal process design a high reactor loading and a mixed flow character seem profitable.

SYMBOLS AND ABBREVIATIONS

- \(C\): concentration (kg/m\(^3\) or kg/m\(^3\) alginate)
- \(D_e\): effective diffusion coefficient (m\(^2\)/s)
- \(D_t\): total dilution rate (hr\(^{-1}\))
- \(1-\varepsilon\): fraction alginate beads (m\(^3\) alginate/m\(^3\))
- \(\phi\): generalized modulus (-)
- \(\Phi_v\): volumetric flow rate (m\(^3\)/hr)
- \(k\): ratio butanol/isopropanol (kg/kg)
- \(K_s\): Monod constant (kg/m\(^3\))
maintenance coefficient (kg/kg D.W. hr)

\( \mu \) specific growth rate (hr\(^{-1} \))

\( n \) polynomial degree or order of reaction (-)

\( \varepsilon \) effectiveness factor (-)

\( r \) reaction rate (kg/m\(^3\) hr or kg/m\(^3\) alginate hr)

\( R \) or \( r \) sphere radius (m)

RSS residual sum of squares

Re dimensionless Reynolds number (-)

Sc dimensionless Schmidt number (-)

Sh dimensionless Sherwood number (-)

\( V_t \) total volume (m\(^3\))

\( Y_{sb} \) butanol yield factor (kg/kg)

\( Y_{sx} \) biomass yield factor (kg D.W./kg)

Subscripts:

b bulk phase

S substrate

B butanol

SO substrate, feed

i interface

X biomass

IP isopropanol

XL biomass in liquid phase

max maximum

XS biomass in solid phase

P product

LITERATURE


paper IV

A simple model for the continuous production of butanol by immobilized Clostridia.

Part II: Cl. species on glucose and whey permeate.

A SIMPLE MODEL FOR THE CONTINUOUS PRODUCTION OF BUTANOL BY IMMobilIZED CLOSTRIDIA.
Part II: Cl.species on glucose and whey permeate.

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SUMMARY
The kinetic model developed for the continuous production of butanol by immobilized Clostridia, was tested for Clostridium species DSM 2152, immobilized in calcium alginate beads. The model described the butanol production rates adequately both on a model substrate, glucose, and on a technical substrate, whey permeate. For both substrates the same (about 55 kg substrate/m³ alginate.hr) and the maximal butanol concentration, C_B_max, was much lower on whey permeate media than on glucose media (4.7 kg/m³ and 7.4 kg/m³). The operational stability of the immobilized Clostridium species was good: experiments lasted for 1000-1800 hours without loss of activity and without disruption of the calcium alginate beads.

INTRODUCTION
In the first part of this study a simple model was proposed to describe the production of isopropanol-butanol mixtures by immobilized Clostridia as a function of the most important process conditions. This model was derived from data on glucose fermentations by Clostridium beyerinokii LMD 27.6, immobilized in calcium alginate beads. The aim of the second part of this study is to demonstrate the validity of the model for another butanol producing strain (Clostridium species DSM 2152) and to show that the model can also be used to describe the production on a technical substrate: whey permeate.

An economic evaluation of a large scale continuous butanol production process with immobilized Clostridia showed that whey permeate can be an interesting substrate for this fermentation.

In order to predict reactor performances under various process conditions with a technical substrate, kinetic models developed for model substrates have to be adjusted to the technical substrate. The applicability of the model for different Clostridium strains would allow a direct reactor performance calculation under various conditions (for example to optimize the process) once 2 or 3 parameters of the strain of interest on the substrate of interest are known.

In this study Clostridium species DSM 2152 was used, an undefined Clostridium strain producing butanol, isopropanol, trace amounts of acetone and butyrate and acetate. Glucose, lactose and whey permeate were used as carbon sources.
**THEORY**

The simplest model found to adequately describe the steady state butanol (C), isopropanol (CIP) and residual carbohydrate (Cg) concentrations in a continuous culture of immobilized Clostridia as a function of the feed substrate concentration (Cg), the dilution rate (D) and the biocatalyst fraction (1-ε) is presented by the equations 1, 2 and 3.

\[ \frac{D_t(C_g - C_p)}{(1-\epsilon)} = \eta r_{max} \left(1 - \frac{C_B}{C_{B,max}}\right) \]  

(1)

\[ C_B = Y_{sb}(C_g - C_p) \]  

(2)

\[ C_{IP} = C_B/k \]  

(3) CIP = C B / k

The validity of the model equations for the new strain (as well as for a different substrate) can be tested by rearranging equations 1, 2 and 3 to equation 4.

\[ \frac{D_t C_B}{(1-\epsilon)} = \frac{Y_{sb} D_t r_{max} C_{B,max}}{1 - \frac{C_B}{C_{B,max}} + \frac{r_{max}}{Y_{sb}} (1-\epsilon)} \]  

(4)

In this rearranged equation η was left out as it was already shown that η is unity for nearly all cases.

The left hand term of equation 4 equals the specific butanol production rate, denoted as \( \frac{P}{Y_{sb} r_{max}} \), in kg butanol/m^3 alginate/hr.

The reciprocal of equation 4 leads to equation 5:

\[ \frac{1}{P} = \frac{1}{Y_{sb} r_{max}} + \frac{1}{C_{B,max} D_t (1-\epsilon)} \]  

**MATERIALS AND METHODS**

*Clostridium species* DSM 2152 was obtained from Dr. Biebl of the GBF in Braunschweig, West Germany. The organism was subcultured at 30 °C with heat shock treatments (1 min. 100 °C, 1 min. 0 °C) for 6-10 times on media containing 60 kg/m glucose (Baker, The Netherlands), 10 kg/m yeast extract (Difco) and 5 kg/m CaCO3. Prior to immobilization spores were harvested from 20 l. batch cultures at 30 °C on 60 kg/m glucose and 10 kg/m yeast extract (Difco).

The spores were immobilized by dissolving 60 kg/m wet spore paste in distilled water and adding sodium alginate (Manucol LD, All, Great Britain) to a 80 kg/m alginate solution. The immobilization procedure described earlier was used, producing calcium alginate beads of 2 x 10 mm diameter.

All continuous experiments were carried out in CSTR's (Stirring speed 100-200 rpm) with working volumes of 0.4 l. at 30 °C. (Experimental set-up described elsewhere). During the start-up phase of all experiments a medium containing glucose (30 or 50 kg/m^3), yeast extract (10 kg/m^3) and CaCl2·2H2O (5 kg/m^3, Merck) was used.
The pH at t=0 varied between 5.6 and 6.0 (medium pH) and was not controlled during the experiment. The dilution rate during the start-up phase varied between 0.07 and 0.1 hr⁻¹, but was always below 0.5 * (1-\(e\)), a value found to give the best results during start-up. After the start-up phase (first 24–48 hrs) the dilution rate was increased to the desired value (data on low dilution rates were obtained by lowering the dilution rate from a higher level, rather than leaving the culture at the start-up dilution rate).

After stabilization of the fermentation, glucose, if desired, was substituted for either lactose (50 kg/m³) (Baker, The Netherlands) or whey permeate (50 kg/m³) (NIZO, The Netherlands). The permeate was obtained in dried form and contained ± 84% lactose (dry solids base). All media were supplemented with 10 kg/m³ yeast extract and 5 kg/m³ CaCl₂·2H₂O sterilized separately at 110 °C and mixed at room temperature. Liquid samples were taken at regular intervals, the frequency was depending on the dilution rate. Glucose concentrations were determined enzymatically, lactose concentrations by HPLC and concentrations of the main products (butanol, isopropanol) and the by-products (acetate, butyrate) by gaschromatography (Conditions described elsewhere).

RESULTS

The steady state concentrations of the fermentation products were measured as a function of the dilution rate for both the model substrate glucose and the technical substrate whey permeate.

Glucose fermentations

The steady state results of the experiments on glucose media are shown in table 1.

Table 1: Steady state concentrations on glucose media as a function of \(D_t\) and \((1-e)\). (Total acids concentrations below 1 kg/m³).

<table>
<thead>
<tr>
<th>(D_t) (hr⁻¹)</th>
<th>((1-e)) (m³alg/m³)</th>
<th>(C_B) (kg/m³)</th>
<th>(C_{IP}) (kg/m³)</th>
<th>(C_{SO}) (kg/m³)</th>
<th>(\Delta C_S) (kg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.12*</td>
<td>0.37</td>
<td>6.0</td>
<td>3.4</td>
<td>N.D.</td>
<td>-27</td>
</tr>
<tr>
<td>0.14</td>
<td>0.37</td>
<td>5.8(5%)</td>
<td>3.4</td>
<td>N.D.</td>
<td>-26</td>
</tr>
<tr>
<td>0.18</td>
<td>0.33</td>
<td>6.0(6%)</td>
<td>2.25</td>
<td>49.8</td>
<td>26.8</td>
</tr>
<tr>
<td>0.19</td>
<td>0.37</td>
<td>5.2(4%)</td>
<td>2.3</td>
<td>50.0</td>
<td>24.1</td>
</tr>
<tr>
<td>0.26</td>
<td>0.35</td>
<td>5.5(3%)</td>
<td>3.2</td>
<td>30.2</td>
<td>27.4</td>
</tr>
<tr>
<td>0.34</td>
<td>0.35</td>
<td>5.1(3%)</td>
<td>2.9</td>
<td>28.7</td>
<td>23.8</td>
</tr>
<tr>
<td>0.76</td>
<td>0.35</td>
<td>2.6(6%)</td>
<td>1.1</td>
<td>23.8</td>
<td>10.9</td>
</tr>
</tbody>
</table>

Note: * Measured for less than 8 residence times.

From table 1 the values for the specific butanol production rate, \(P = D_t C_B/(1-e)\) were calculated. Figure 1 shows a double reciprocal plot of \(P_B\) versus \(1/(1-e)\).

The points in this plot can be described with a straight line (corr. coeff. = 0.982) with slope 0.135 and intercept 0.084.

From the theory section it is recalled that the slope of the line equals \(1/C_B,\text{max}; C_B,\text{max}\) is therefore estimated to be 7.4 kg/m³. The intercept is formed by \(Y_{sb} r_{\text{max}}\). From table 1 the mean \(Y_{sb}\) value is calculated to be 0.22. This leads to an estimation for \(r_{\text{max}}\) of 54 kg glucose/m³ alginate.hr.

The last parameter, \(k\), can be deduced from table 1 by calculating the average \(C_B/C_{IP}\) ratio. Its value is approximately 2.
Figure 2: Double reciprocal plot of $P$ vs. $D_t/(1-\varepsilon)$. Independent experiments are represented by different symbols (+ is the lactose experiment). ($1/P$ in $m^3$ alginate.hr/kg butanol and $(1-\varepsilon)/D_t$ in $m^3$ alginate.hr/m$^3$).

Table 3: Parameter values determined from continuous experiments with immobilized Clostridium species DSM 2152 on glucose and whey permeate.

<table>
<thead>
<tr>
<th></th>
<th>glucose</th>
<th>whey permeate</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_{max}$</td>
<td>54</td>
<td>55</td>
</tr>
<tr>
<td>($kg$/substrate/$m^3$ alginate.hr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{B,max}$</td>
<td>7.4</td>
<td>4.7</td>
</tr>
<tr>
<td>($kg$/m$^3$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Y_{sb}$</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>($kg$/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k$</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>($kg$/kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The $C_{B,max}$ values, representing the maximum inhibiting butanol concentration, are lower for whey permeate fermentations than for glucose fermentation. In the literature it was recently reported that a similar phenomenon was observed for butanol inhibition of *Clostridium acetobutylicum* in xylose and glucose fermentations. The product inhibition was found to be stronger for xylose, hypothetically due to a membrane bound substrate transport mechanism that was active in the case of xylose and not in the case of glucose. As butanol can affect the cell membrane, it could influence the xylose transport more strongly than the non-active glucose transport. It is possible that this also holds for butanol inhibition in immobilized *Clostridium* cells on lactose media. As no free galactose or glucose were detected on lactose media, the lactose could very well be transported into the cell via an active transport mechanism.

From the lactose/permeate experiments it can also be concluded that at the $C_{50}$ levels and $D_t$ values observed no large differences in performance were found between whey permeate and lactose.
Figure 3: Comparison of the model predictions for glucose and whey permeate for immobilized Clostridium species.

This means that neither the additional salts nor the protein residues in the permeate interfere severely with the fermentation. (At extremely high permeate concentrations salts inhibition could occur).

From an operational point of view it is important to notice the overall higher butanol concentrations found with Clostridium species, compared with Clostridium beyerinckii. Also, Clostridium species appears to be very stable in an immobilized form. Various experiments reported here ran for 1000-1800 hours without loss of activity (productivities observed matched model predictions). After these long periods of operation the alginate beads although showing signs of wear, were still intact. This shows that hardening of the beads which is often encountered in the literature (chemically or by drying) is not necessary for the type of alginate used and the strain immobilized, if sufficient Ca²⁺-ions are present in the fermentation broth. Furthermore it was found that the medium requirements for Cl. species are not so stringent as for Cl. beyerinckii. Yeast extract concentrations of 5 kg/m³ were found to be sufficient and led to the same results as 10 kg/m³ yeast extract. The concentration can probably be lowered even more, especially when whey permeate is used as a carbon source.

A further optimization, especially in the area of strain properties is necessary for large scale application.
CONCLUSIONS

The kinetic model, originally developed for Clostridium beyerinckii (immobilized in calcium alginate beads) on glucose media, can also be used to satisfactorily predict the butanol production rates of immobilized Clostridium species DSM 2152 as a function of the process conditions. Furthermore, the model can be used for both fermentations on the model substrate glucose and for fermentations on the technical substrate whey permeate. For both substrates the $r_{\text{max}}$ values are about 55 kg/m$^3$ alginate.hr; overall butanol yields are comparable (0.22 kg/kg), but the $C_{B,\text{max}}$ values for whey permeate fermentations are substantially lower than for glucose fermentations (4.7 kg/m$^3$ and 7.4 kg/m$^3$ respectively). Immobilized Clostridium species cells are extremely stable: continuous experiments lasted for 1000-1800 hours without loss of activity.

LITERATURE

7. C. Frick, K. Schügerl, Vortrag Achema 85, Internationales Treffen für Chemische Technik.

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LIST OF SYMBOLS

- $C_b$: butanol concentration
- $C_{B,\text{max}}$: maximal butanol concentration
- $C_{IP}$: isopropanol concentration
- $C_s$: substrate concentration
- $C_{S0}$: substrate feed concentration
- $\Delta C_s$: consumed substrate concentration
- $D_t$: dilution rate based on total volume
- $k$: butanol/isopropanol ratio
- $P$: specific butanol production rate
- $r_{\text{max}}$: maximal specific substrate consumption rate
- $Y_{sb}$: butanol yield on substrate
- $(1-c)$: biocatalyst fraction
- $\eta$: effectiveness factor
- $D_t/(1-c)$: normalized total dilution rate

kg/m$^3$ alginate.hr

kg/kg

m$^3$ alginate/m$^3$

m$^3$ alginate/m$^3$

kg substrate/m$^3$ alginate.hr

kg/kg

kg butanol/m$^3$ alginate.hr
A comparative study of a fluidized bed reactor and a gas lift loop reactor for the IBE process.

Part I: Reactor design and scale down.

Accepted for publication in: The Journal of Chemical Technology and Biotechnol, 1986.
A COMPARATIVE STUDY OF A FLUIDIZED BED REACTOR
AND A GAS LIFT LOOP REACTOR FOR THE IBE PROCESS
Part I: Reactor design and scale down approach.

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SYNOPSIS
For the continuous production of isopropanol-butanol mixtures by immobilized Clostridia (the IBE process) two reactor types were studied: a fluidized bed reactor with liquid recycle (FBR) and an external loop gas lift reactor (GLR). A large scale design (50-65 m³) was made for both reactors. A regime analysis, by evaluating the time constants for e.g. mixing and conversion, identified the ruling regime. Via the scale down approach two representative model reactors were developed: a 10 liter FBR reactor (H/D=25, D=0.08 m) and a 15 liter external loop GLR (H/D=12.5, D=0.08 m). For both reactors the hydrodynamical behaviour and the total reactor performance were studied and described in parts II and III of this study.

INTRODUCTION
Application of immobilized cells in continuous operation to obtain high reactor productivities and long term stability is more often encountered in the literature. The Kyowa Hakko Kogyo Co 1 has had a pilot plant reactor operating since 1982 to produce ethanol continuously with yeasts immobilized in calcium alginate beads. A significant increase in productivity (compared with a suspended cell culture) and a 6 months steady production were reported; no data concerning the economical feasibility of this particular application of immobilized cells were included however. Production of higher alcohols can be achieved by using Clostridia anaerobic bacteria that can ferment various carbohydrates to butanol and isopropanol (or acetone), CO₂ and H₂.

A recent study on the isopropanol/butanol production by Clostridia immobilized in calcium alginate beads showed that continuous operation of the process was possible with relatively long term stability (appr. three months) - both on a model substrate (glucose) and on a technical substrate (whey permeate) 2-4. A preliminary costing study of the process revealed that a large scale realization of this application of immobilized cells may become economically feasible 5.

Aim of the three part study presented here is to compare two reactor types possibly suitable for the large scale application of the butanolic fermentation with immobilized Clostridia (IBE process). The procedure used was to select two reactor types, design full scale reactors and develop representative model scale reactors. This (mostly theoretical) part of the study is described in part I.

Secondly in both reactors the transport phenomena were studied to describe and predict the hydrodynamic behaviour of the 3-phase system with sufficient accuracy. Part II covers this section of the study as well as the development of integrated reactor models. Thirdly the total reactor performances and operational aspects were studied by carrying out fermentations with immobilized Clostridia in the reactors. The experimental data were compared with predictions derived from the integrated reactor models. This concluding section is described in part III.

REACTOR CHOICE
A previous, kinetic study on the immobilized Clostridia showed that a relatively simple model, using linear butanol inhibition kinetics, can be used to adequately describe the production of isopropanol and butanol from either glucose or whey permeate in a continuous system. 3, 6 Product inhibition generally speaking favours a plug flow character. However, in the literature it was described that a well mixed character is essential for germination of the immobilized biomass. 6 The fact that CO₂ and H₂ are produced during the fermentation, along with the main products butanol and isopropanol, favours a reactor with easy gas release. The kinetic study also revealed that a high fraction of alginate beads in the reactor is advantageous, because both the reactor productivity and the product concentrations increase with increasing reactor loading.
Four reactor types suitable for large scale use with a three phase system (biocatalyst, broth, gas) were considered and quantitatively evaluated for their applicability in the large scale i-propanol-butanol process (IBE process).

**Continuously stirred tank reactor (CSTR):** From laboratory scale data the obtainable solids hold up is estimated to be 30%. Due to the shear sensitiveness of the calcium alginate beads, low stirrer speeds are required on a large scale to prevent high bead attrition rates. However, these low stirrer speeds are likely to cause settling of the beads at the bottom of the reactor (see appendix for calculation), thus hindering escape of the fermentation gas. This forms a serious drawback for application of the CSTR in the IBE process on production scale.

**Packed bed column reactor (PBR):** High solid hold up values (50-60%) are obtainable in this reactor type. Disadvantageous for a large scale use in the IBE process is the difficult release of fermentation gas (accumulation of gas in the bed will cause slugs and create zones inaccessible to substrate). Furthermore the plug flow character is unfavourable for the biomass development.

**Fluidized bed reactor (FBR):** In a fluidized bed reactor with or without liquid recycle the biocatalyst fraction will approximately be 40-45%. For a FBR without liquid recycle, the substrate inlet flow completely determines the fluidization velocity; this will lead to FBR reactors with a plug flow character (disadvantageous for starting up) and very high H/D ratios to obtain the desired conversion. Fluidization of the alginate beads can also be achieved by circulation of the fermentation broth. The degree of mixing in the reactor will be considerable, mainly due to the mixing obtained by the recycle loop. Gas release is expected to be easy (loose structure of the fluidized bed). Attrition rates will be lower than in a CSTR, due to the absence of mechanical mixers.

**Gas lift loop reactor (GLR):** An external loop reactor was considered as an alternative for the FBR in search for a well mixed, non stirred reactor. The GLR typically consists of a riser column and a downcomer column. The driving force for the liquid circulation in the reactor is obtained by creating a difference in gas hold up between the riser and the downcomer.

For the IBE process this can be achieved by recirculating the produced fermentation gas through the riser column. Release of the produced gas will not be problematic (high superficial liquid and gas velocities). An external as opposed to an internal loop reactor was considered because of a more regular and better defined flow pattern and the better degassing at the top of the reactor. Reactor loadings of 20-40% have been reported in the literature. Again, attrition rates are expected to be lower than in a CSTR. Considerations concerning obtainable reactor productivities, biocatalyst attrition rates and operational stability led to the conclusion to study the fluidized bed reactor with liquid recycle and the external loop gas lift reactor more closely for their large scale applicability in the IBE process.

**SCALE DOWN APPROACH**

To develop and optimize a process experiments are often carried out on laboratory scale. By means of the scale down approach a process or reactor can be studied on laboratory scale in a way that is representative for and translatable to the production scale. First the reactor is designed on production scale in a paper study; such a design is based on available models predicting transport phenomena etc. on a large scale. Often these models are mixtures of empirical relations and rules of thumb. Then the designed process can be scaled down to laboratory scale. This can be achieved by means of a regime analysis, using the time constants of the various relevant mechanisms in the reactor during steady state, continuous operation. (Time constants are inversely proportional to the rate of a process, time constants can be defined for substrate consumption, mixing, mass and heat transfer, etc.) Once the rate determining regime is established on production scale, this has to be simulated on laboratory scale. Optimization of the process on laboratory scale can then lead to an optimized design for the production scale.

**Fluidized bed reactor:** Figure 1 schematically depicts the fluidized bed reactor with liquid recycle. Before a realistic design of the production scale reactor can be made, its size has to be determined. Not many literature data on three phase liquid fluidized systems are available, but some reactors
Figure 1: Fluidized bed reactor with liquid recycle.

are reported varying in size from 1 to 225 m$^3$. A rather arbitrary choice of 50 m$^3$ was made for the size of the production scale FBR. The size is large enough to treat industrially realistic amounts of whey permeate, a possible substrate for this process$^{1,2,4,5}$. Next, the H/D value of the production scale reactor is to be defined. A low H/D value is advantageous for gas release, too low a H/D value is unfavourable because a regular fluidization pattern will then be difficult to maintain. The H/D ratio was set at 3 (the literature reports values of 2.5-4)$^{12}$. This leads to a 50 m$^3$ FBR, $H=8.3$ m and $D=2.8$ m. The diameter of the biocatalyst particles (alginate beads containing the micro-organisms), was chosen at 2.0×10$^{-3}$ m, which is the laboratory scale value.$^2$ The fraction of alginate beads was chosen to be 45%.

A regime analysis was carried out for the 50 m$^3$ FBR, assuming that the hydrodynamic behaviour of the reactor approximates that of a two phase liquid fluidized bed. This assumption is necessary to obtain order of magnitude estimations of liquid velocities and axial dispersion coefficients. It seems valid because the gas phase consists of in-situ produced gas bubbles which are not expected to exceed diameters of 1.0×10$^{-3}$ m. Literature data$^6$ show that very small gas bubbles (<1.0×10$^{-3}$ m) are likely to behave as rigid spheres (hardly coalescing) and rise steadily. These small bubbles are not expected to greatly influence the liquid flow pattern.

Time constants:

The fermentation rate can be expressed by the time constant for production:

$$t_p = -\frac{C}{r_c}$$

with $t_p$ = time constant for production (s)

$C$ = concentration (product) (kg/m$^3$)

$r_c$ = production rate (kg/m$^3$.s)

In a mixed reactor in steady state, no accumulation of product in the reactor will be found, therefore:

$$r_c = D_t \ast C$$

with $D_t$ = total dilution rate (s$^{-1}$)

The developed kinetic model$^3,4$ shows for a well mixed reactor, with a beads fraction of 45% that a total dilution rate of 0.3-0.5 hr$^{-1}$ leads to reasonable product concentrations and production rates. Substitution of this estimated $D_t$ range into equations (2) and (1) leads to an estimated time constant for production:

$$t_p = 7.2\times10^{-3} - 1.2\times10^{-4}$$

The degree of mixing in the reactor can be estimated from the axial dispersion in the fluidized bed and the mixing as a result of the recycle loop. This mixing due to circulation can be expressed by the time constant for circulation; this time constant is approximated by equation (4):

$$t_{circ} = \frac{\phi_{circ} \times e \times V}{c \times \phi_{circ}}$$

with $t_{circ}$ = circulation time (s)

$V$ = reactor volume (m$^3$)

$e$ = liquid fraction

$\phi_{circ}$ = volumetric flow rate (m$^3$/s)

A volumetric balance over the reactor leads to an estimation of $\phi_{circ}$:

$$\phi_{circ} = \frac{V_1 \ast A - D_t \ast V}{V}$$

with $V_1$ = superficial liquid velocity (m/s)

$A$ = reactor surface area (m$^2$)

Richardson and Zaki$^{1,4}$ derived a method to estimate the $V_1$-value in a liquid fluidized bed, as a function of the solids hold up, particle diameter and particle density.
With a density of 1050 kg/m³ for alginate beads and the already defined values for loading and particle diameter, a superficial liquid velocity of \( 5 \times 10^{-3} \) m/s was estimated for the 50 m³ FBR. With the reactor diameter of 2.8 m this then leads to a circulation time of:

\[
t_{c_{irc}} = 600-1200 \text{ s.}
\]

The **axial dispersion time** is defined as:

\[
t_{ax} = \frac{n^2}{D_{ax}}
\]

with \( t_{ax} \) - time constant for axial dispersion (s)

\( n \) = height (m)

\( D_{ax} \) = axial dispersion coefficient (m²/s)

As no dispersion coefficients for beads of comparable density, measured in large scale fluidized beds were found in the literature, an order of magnitude estimation was made from laboratory scale data, presented by Kikuchi et al. An axial dispersion coefficient of \( 10^{-4} \) to \( 10^{-5} \) m²/s was found for polystyrene beads (density 1050 kg/m³) of comparable diameter in a liquid fluidized bed column of \( 1.0 \times 10^{-3} \) m at \( v_l = 10^{-2} \) to \( 10^{-3} \) m/s. For the 50 m³ FBR the axial dispersion time, with a reactor height of 8.3 m was estimated to be:

\[
t_{ax} = 6.9 \times 10^5 - 6.9 \times 10^6 \text{ s}
\]

The time constants for diffusion and reaction in the calcium alginate matrix were considered to be of no importance for the regime analysis: in CSTR reactors it was already found that the effectiveness factors for this particular process are near unity, which means that the diffusion process is faster than the reaction process in the matrix. In the 50 m³ FBR, effectiveness factors are expected to be in the same order of magnitude (same range of substrate, product and immobilized biomass concentrations), therefore these time constants do not have to be included explicitly in the regime analysis. Evaluation of the time constants for axial dispersion and circulation in the 50 m³ FBR shows that the circulation time is much smaller than the dispersion time, therefore mixing is mainly a result of circulation. Furthermore the circulation time is much smaller than the production time, but not so small that the 50 m³ bioreactor can be considered entirely mixed. It is important to maintain the same regime on laboratory scale.

Figure 2 shows estimated values of \( t_{c_{irc}}, t_{p}, t_{ax} \) as a function of reactor volume for various H/D ratios (solids hold up, \( v_l \) and \( D_{ax} \) constant for all volumes).

From this figure it can be seen that with decreasing reactor volume, H/D has to increase to resemble the production scale reactor as closely as possible. On 10 liter scale an H/D ratio of 25 still shows the same ratio of \( t_{c_{irc}}/t_{ax} \) compared with \( t_{p} \); the degree of mixing is therefore expected to be comparable to that of the 50 m³ FBR, as well as the concentration gradients. The superficial gas velocity, \( v_g \), which was not taken into account in the above mentioned, will be a function of the bed height, as gas is produced in-situ and is not recirculated. To simulate the production scale gas velocities, the model reactor formally has to be as high as the production reactor (8.3 m).
The diameter of the model reactor should be large enough to avoid wall effects; as a rule of thumb 10-20 times the bead diameter was taken as a minimum. Ultimately, the model reactor chosen was a 10 liter fluidized bed reactor H/D=25, H= 2.0 m, D=0.08 m. To study the influence of the fermentation gas on the hydrodynamics in the production scale reactor, extra gas can be added to the inlet of the model reactor (compensation for the height), although this will remain an approximation.

Gas lift loop reactor: Figure 3 schematically presents the external loop GLR. In order to compare the performances of the FBR and GLR, a production scale size of 65 m$^3$ was chosen for the GLR (same total production). Most literature data concern air lift systems$^{9,17,18,20}$; design rules used for these air lifts are mainly based on superficial gas velocities derived from oxygen demands and are not useful for the IBE process, in which mixing forms the most important design criterion. With respect to mixing times it is reported$^9$ that these become lower at decreasing H/D ratios. Decreasing riser to downcomer ratios (approaching unity) also seem to improve the degree of mixing. For the 65 m$^3$ GLR a H/D ratio of 5 was chosen and $D_r/D_d$ was set at 1, H=10 m, $D_r=D_d=2.0$ m. A reactor loading of 35% was estimated to be reasonable.

Contrary to the FBR, the gas phase in the GLR will determine to a large extent the hydrodynamical behaviour of the reactor (extra gas is introduced, which leads to far higher superficial gas velocities). As an approximation, literature data on two phase (G-L) bubble columns and loop reactors were used to obtain estimations of the various time constants in the 65 m$^3$ GLR (small density difference between liquid and solid phase).

Time constants:
The time constant for production was calculated from equations (1) and (2), using the kinetic model$^{19}$ to estimate $D_t$. At a solids hold up of 35% a dilution rate range of 0.2-0.4 hr$^{-1}$ is found for specific production rates, comparable to those estimated for the fluidized bed reactor. Therefore,

$$t_p = 9.0 \times 10^3 \text{ - } 1.8 \times 10^4 \text{ s.}$$

The circulation time was calculated using equation (6), neglecting the gas hold up.

$$t_{circ} = \frac{v}{v_{1} + \frac{A_r - D_t dV_l}{D_t dV_l}}$$

with $A_r$ = riser surface area (m$^2$).

For the axial dispersion time equation (7), analogous to equation (5) was used:

$$t_{ax} = \frac{L^2}{v_{ax}^2}$$

with $L$ = total reactor length (m).

For aerated loop reactors, superficial gas velocities of 1.0x10$^{-1}$ m/s are reported$^9$. For the GLR a superficial gas velocity range of 1.0x10$^{-2}$-3.0x10$^{-2}$ m/s was thought to be reasonable.

Joshi presents correlations to predict superficial liquid velocities from superficial gas velocities in bubble columns$^{19}$. For the above mentioned $v_g$ values this would lead to superficial liquid velocities in the range of $0.5-1.0$ m/s in the 65 m$^3$ GLR. Onken and Weiland$^{20}$ report liquid velocities of 1.0-2.0 m/s in an industrial size deep shaft reactor. This leads to a circulation time of:

$$t_{circ} = 20-40 \text{ s.}$$
From reviewed data Joshi composed an equation to calculate liquid axial dispersion coefficients from superficial gas velocities; applied to the GLR this leads to:

\[ D_{ax} = 0.33 \left( g D_r v \right)^{1/3} \]  

with \( v \) = superficial gas velocity (m/s)
\( g \) = gravitational constant (m/s²)

Substitution of equation (8) into equation (7) leads to:

\[ t = \frac{700-1000}{\text{ax}} \text{s} \]

As for the FBR, the time constants for diffusion and reaction in the immobilization matrix were considered to be of no importance for the regime analysis.

The estimations of the time constants in the 65 m³ GLR show that both the axial dispersion time and the circulation time are lower than the production time. For a laboratory scale (10 l) reactor of various geometries (H/D=10 and 5; D_r/D_d = 2 and 1) the time constants were found to be: t_ax = 60-500 s., t_circ = 5-20 s., t_p = 9.0×10³-1.8×10⁴ s.

This means that no change of the ruling hydrodynamical regime is likely to occur when scaling down to laboratory scale. Furthermore, the rather arbitrary choice of H/D=5 and D_r/D_d = 1 for 65 m³ scale is of no great influence on the model design. The one criterium that determines the design of the model reactor is the minimum requirement for riser and downcomer diameter to avoid beads' and bubbles' wall effects. The model external loop GLR chosen was a reactor of 15 liter volume, H=1.0 m, D_r=D_d=0.08 m and B (width bottom section) is 0.5 m. From literature data it cannot be concluded whether wall effects concerning the gas bubbles will be observed at this riser diameter (enhanced coalescence of the sparged gas bubbles is likely to occur). This particular effect will have to be studied closely in the model before it can be established whether valid conclusions for the production scale gas behaviour in the GLR can be drawn.

Parts II and III of this study deal with hydrodynamics and total reactor performance of the two reactor types respectively.

CONCLUSIONS

A fluidized bed reactor with liquid recycle (FBR) and an external loop gas lift reactor (GLR) were chosen to study more closely for a possible large scale application in the IBE process: the continuous production of isopropanol/butanol mixtures with immobilized Clostridia. For both reactors a production scale design was made from literature data. Model reactors for both systems were designed starting from the production scale reactors using the scale down approach (A regime analysis was made by evaluating the time constants of the important processes in the reactors). A 10 liter FBR model reactor was developed (H/D=25, D_r=0.08 m) as well as a 15 liter GLR model reactor (H/D=12.5, D_r=D_d=0.08 m, B=0.5 m) to study the hydrodynamics of the three phase IBE process (part II) and the total reactor performances and operational stabilities (part III).

REFERENCES

APPENDIX

Laboratory scale data report stirrer speeds of 200 rpm in a 0.6 l CSTR (impeller diameter 0.04 m) filled with 30% alginate beads. Calcium alginate beads hardened by drying are mechanically more stable than undried ones, but stirrer speeds of 500 rpm are given as the upper limit of 1-2 l CSTR's (impeller diameter approx. 0.04 m). Wang et al. describe a method to use constant shear as a scale-up criterion. Shear rates are found to be proportional to impeller tipspeeds (ND). From equation (1a) the stirrer speed for production scale can be estimated when the above mentioned stirrer speed of 500 rpm is taken as an operational value.

\[
\text{(ND)}_{\text{lab. scale}} = \frac{\text{(ND)}_{\text{prod. scale}}}{N^{\text{lab. scale}}} \quad \text{(1a)}
\]

Therefore:

\[
N_{\text{prod. scale}} = N_{\text{lab. scale}} \left( \frac{D_{\text{lab. scale}}}{D_{\text{prod. scale}}} \right)^{3/2} \quad \text{(2a)}
\]

For a 65 m³ CSTR (H-T=4.4 m, D=731x1.45 m) this then leads to an operational stirrer speed of 0.09 s⁻¹ and an upper limit of 0.23 s⁻¹, which is extremely low. For production scale it is of importance to keep the calcium alginate beads suspended so that gas escape is facilitated. From literature data it was estimated what the critical stirrer speed should be to keep the alginate beads in suspension.

Equation (3a) leads to this critical value, \( N_c \).

\[
N_c = 0.22 \text{ s}^{-1}. \quad \text{Therefore, } N_c \text{ is 0.22 s}^{-1}. \text{ This estimation shows that the stirrer speed necessary to keep the beads in suspension is even higher than the stirrer speed desirable from shear stress considerations and will most likely cause high bead attrition rates.}
\]

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paper VI

A comparative study of a fluidized bed reactor and a gas lift loop reactor for the IBE process.
Part II: Hydrodynamics and reactor modelling.

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A COMPARATIVE STUDY OF A FLUIDIZED BED REACTOR AND A GAS LIFT LOOP REACTOR FOR THE IBE PROCESS

Part II: Hydrodynamics and reactor modelling

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SYNOPSIS

A fluidized bed reactor with liquid recycle (FBR) and an external loop gas lift reactor (GLR) were designed for the production of isopropanol-butanol mixtures by immobilized Clostridia and scaled down to laboratory scale (part I). Hydrodynamic models were set up for the two laboratory scale reactors. Liquid mixing in the 10 l. FBR was described by 10 tanks in series. Fluidization velocities, bed expansions and axial dispersion coefficients agreed well with literature data. Liquid mixing in the 15 l. GLR was described by 100 tanks in series. The gas hold up and circulation velocity were found to decrease with increasing solids hold up, in accordance with literature indications. No influence of the solids hold up on the axial dispersion coefficient was determined. An integrated reactor model was set up for both reactors, using the hydrodynamic and kinetic model. Actual fermentation data are presented and compared with model predictions in part III of this study; this part will also include a comparison of reactor performances and scale up aspects.

INTRODUCTION

A study was made of two reactor types selected for the production of isopropanol-butanol mixtures in a continuous fermentation using Clostridia immobilized in calcium alginate beads. In part I of this study a 50/65 m³ design was made of both reactors- a fluidized bed reactor with liquid recycle (FBR) and an external loop gas lift reactor (GLR).

THEORY

The degree of liquid mixing in both reactors can be described by a tanks in series model.

Pulse response measurements can be used to obtain the dimensionless group $\frac{v \cdot L}{D_{ax}}$ and the number of tanks. Explicit calculation of $v$ and $D_{ax}$ allows a direct comparison with literature data using dispersion models. Once the number of tanks is known, the hydrodynamic model can be combined with the previously developed kinetic model (summarized in appendix A) and,
completed with an overall mass balance to account for the liquid recycle, this results in an integrated reactor model. The overall structure of the integrated model is presented in a block diagram (figure 1). The upper part of the diagram shows the three parameters that mainly determine the liquid mixing in the reactor: $D$, $v$, and $L$. It is discussed in the following which correlations were used to obtain these parameters for each reactor.

\[
\begin{align*}
\frac{d v}{d t} &= f(d_p, \rho_s) \\
\epsilon_s &= f(d_p, v) \\
\phi_R &= f(v, \epsilon_{in}) \\
N &= f(v, D_{ax}, L) \\
\phi_R &= f(v, \epsilon_{in}) \\
V_t &= f(H, D) \\
V_{in} &= f(\tau, N, C_{out})
\end{align*}
\]

Figure 1. Structure of the integrated FBR and GLR reactor models (see also list of symbols).

**Fluidized bed reactor.**

In the fluidized bed reactor the gas phase will consist of in-situ produced gas bubbles, initial bubble sizes and gas hold ups are expected to be low. In the literature it is shown that small gas bubbles (e.g. $5 \times 10^{-4}$ m diameter with Reynolds number of 1-3 at gas hold ups of 2%) are likely to have regular flow patterns around them and will behave as rigid spheres.

The influence of the gas phase on the liquid mixing in the fluidized bed reactor was therefore neglected as a first approach.

The hydrodynamical behaviour of a liquid fluidized bed reactor is determined by the liquid velocity needed to fluidize the solids. From literature data it was derived that the axial dispersion coefficient (for a given particle density and diameter) mainly depends on the superficial liquid velocity $v_{1}$. This superficial liquid velocity depends on the desired solids hold up ($\epsilon_s$), the terminal (falling) velocity of the solids ($v_{\infty}$) and, implicitly the minimum fluidization velocity ($v_{mf}$). This dependency is expressed by the Richardson-Zaki equation:

\[
v_{1} = \epsilon_{1} \left(\frac{v_{\infty}}{v_{1}}\right)^{n}
\]

with $v_{1}$ = superficial liquid velocity (m/s)  
$v_{\infty}$ = terminal (falling) velocity solids (m/s)  
$\epsilon_{1}$ = liquid hold up  
$n$ = exponent

The terminal particle velocity ($v_{\infty}$), which depends on the sphere diameter, $d_p$ and the density difference between solid and liquid phase, $\rho_s - \rho_l$, can be determined experimentally.

The density difference between solids and liquids is obtained from equations (3) and (4):

\[
\rho_s = \rho_l + \frac{3 C_w \rho_l v_{\infty}^2}{4 d_p g}
\]

\[
C_w = 18.5 / Re_{t}^{3/5}
\]

with $\rho_s$ = solids density (kg/m$^3$)  
$\rho_l$ = liquid density (kg/m$^3$)  
$C_w$ = particle friction factor  
$Re_{t}$ = particle Reynolds number

**Gas lift loop reactor.**

In the GLR the hydrodynamic behaviour is mainly determined by the density difference between riser and downcomer section of the reactor. The riser column (into which gas is sparged) has a larger gas hold up than the downcomer (due to degassing at the top of the reactor) which leads to a density difference, causing the liquid (and solids) to circulate.
Hsu and Duduković developed a model to predict liquid circulation rates for two phase gas lift reactors, using an overall hydrostatic balance in principle the model states that in steady state operation the hydrostatic pressure drop must equal the pressure drop due to friction losses. The balance equation was simplified for the two phase GLR by just taking into account the tubular flow friction losses (approximated by Blasius equation for one phase flow) and the friction losses at the bends, leading to equation (5):

$$v^2 = \frac{1}{2g} \left( \frac{H}{D_r} \times 0.316 \text{Re}^{-0.25} \right) + \frac{H}{D_d} \left(- \frac{1}{D_d} \times 0.316 \text{Re}^{-0.25} \right) + 2(K_w_r + K_w_d)$$

with $\varepsilon = \text{gas hold up}$

$H = \text{column height (m)}$

$v_l = \text{superficial liquid velocity(two phase)}$ ($m/s$)

$D_r = \text{riser diameter (m)}$

$\text{Re} = \text{Reynolds number based on tube diameter}$

$d = \text{downcomer}$

$D_d = \text{downcomer diameter (m)}$

$K_w_r, K_w_d = \text{friction factors for bends}$

For the heterogeneous flow regime the gas hold up can also be calculated from equation (6):

$$\varepsilon_g = \frac{v}{v_g + v_b}$$

with $v = \text{superficial gas velocity (m/s)}$

$v_c = \text{liquid circulation velocity(m/s)}$

$v_b = \text{bubble rise velocity (m/s)}$

An iteration procedure using equations (5) and (6) leads to the circulation velocity $v_c$ as a function of $v_g$ for two phase flow.

The complex three phase flow system in the GLR is difficult to describe mechanistically. As an approximation an attempt was made to derive equations from the two phase flow system described above.

In the three phase flow situation the hold ups are defined as:

Gas: $\varepsilon_g = \frac{v}{v}$

Liquid: $\varepsilon_l = \frac{v_l}{v}$

Solids: $\varepsilon_s = \frac{v}{v}$

The reactor was thought to be divided into two compartments, one representing the gas/liquid phase, the other representing the solid phase. For the gas/liquid phase the volume $V_g + V_l$ was defined as $V_c$, with $V = V_t(1-\varepsilon)$ and the hold ups are $\varepsilon_g = V_g / (V + V_l)$ and $\varepsilon_l = V_l / (V + V_l)$.

Equations (5) and (6) are used to calculate the liquid velocity $v_l$ and the gas hold up $\varepsilon_g$ in the gas/liquid compartment with volume $V_c$. (The tube diameter in Blasius equation is $D*(1-\varepsilon)$ 0.33, obtained from $V_c$.)

The results of these calculations for the gas/liquid phase are transformed to the gas/liquid/solid situation by using volume balances for the reactor contents.

The gas hold up for the three phase situation thus becomes:

$$\varepsilon_g = \frac{v}{v_c} = \frac{v_c}{v_c} \cdot \frac{V_c}{V_c(1-\varepsilon)} = \varepsilon_c \cdot (1-\varepsilon)$$

The circulation velocity for the three phase flow is obtained from:

$$v_c = \frac{1}{4 \pi D^2} v + \frac{1}{4 \pi D^2} v + \frac{1}{4 \pi D^2} v = \frac{1}{4 \pi D^2} (1-\varepsilon) 2/3 v$$

This leads to $v_c = v_c^{1-\varepsilon} 2/3$. In this way the circulation velocity, $v_c$, needed to calculate $v_c / \phi_a x$, and the gas hold up, $\varepsilon_g$, are calculated for the three phase situation as a function of solids hold up, $\varepsilon_s$, and superficial gas velocity, $v_g$.

The axial dispersion coefficient in the GLR mainly depends on the superficial gas velocity and the column diameter. Equation (7), derived by Joshi, from reviewed data for two phase flow, was used to calculate $D_a x$ in the two phase situation.

$$D_a x = 0.33 (g b h v_g)^{1/3}$$

The influence of the third phase (alginate beads) on $D_a x$ is hard to predict.

Experiments were carried out for both reactors, determining vital hydrodynamic parameters and comparing them with literature data.

MATERIALS AND METHODS

The fluidized bed reactor (dimensions shown in figure 2a) consists of a double walled glass column (Schott) with stainless steel top and bottom fittings. The liquid distributor (bottom) consists of a stainless steel perforated plate (10 m perforations, 25 mesh/cm2), necessary to ensure even liquid distribution and prevent radial gradients. Liquid was pumped out of the reactor via a liquid/solid separator (teflon tube with slits).
at the top of the reactor and recirculated (by Watson Marlow peristaltic pump, marprene 12.7*10 m tubing). Characteristic reactor length L=2.0 m. The gas lift loop reactor is shown in figure 2b.

LIQUID RECYCLE

©

PULSE INJECTION PORT

Dr J POROUS

lq, I PLATE

GAS IN

0.5 m

PERFORATED PLATE

Figure 2a: Dimensions and set up of the fluidized bed reactor (FBR) gas lift loop reactor (GLR)

The GLR consists of a glass loop (Schott) with stainless steel fittings at top and bottom of the riser column, as well as a stainless steel middowncomer section. Gas was sparged into the riser column through a teflon porous plate. Characteristic reactor length is 2.5 m.

The calcium alginate beads were produced with a vibrating nozzle, as described in the literature. A suspension of 80 kg/m² sodium alginate (Manucol LD, Alginate Industries) was used to form a jet of sodium alginate drops (pressure 3 bar, frequency 180 Hz, flow rate about 8*10⁻⁶ m³/s, nozzle diameter 0.96*10⁻¹ m), which fell into a stirred solution of 40 kg/m² CaCl₂·2H₂O (Merck). After 24 hours stirring the beads were transferred to a 5 kg/m² calcium chloride solution and stored at 4°C. Average bead diameters were determined using a stereomicroscope. Terminal velocities of the beads were determined in a 5*10⁻¹ m tube in water at 20°C by measuring the path length and time.

RESULTS AND DISCUSSION

From the average diameter of the calcium alginate beads (1.94*10⁻³ m) and the terminal bead velocity (0.0265 m/s) the density of the beads was determined to be 1043 kg/m³ (using equations (3) and (4)).

Fluidized bed reactor: Figure 3 depicts the results of the bed expansion measurements in the two phase (L-S) fluidized bed. The results are in good agreement with the calculated values of the Richardson Zaki model (equation (2), with n=3.02), represented as the drawn line in figure 3. The minimum fluidization velocity of the beads was determined via the commonly used pressure drop method (equation (2), with n=3.02), represented as the drawn line in figure 3.
Figure 3: FBR bed expansion measurements. Liquid hold up as a function of superficial liquid velocity. Experiments (x) and calculated values from Richardson-Zaki (drawn line) are shown.

Figure 4: Axial dispersion measurements as a function of superficial liquid velocity in the FBR. Figure 5 shows the results of the axial dispersion measurements as a function of the superficial liquid velocity and liquid hold ups for the two phase fluidized bed. Literature data show a continuous increase of $D_{Ax}$ with $v_l$; the point at $v_l=4 \times 10^{-3}$ m/s was considered an outlier and was not included in the fitted curve drawn through the measured points. The results agree reasonably well with Kikuchi et al., who reported slightly lower $D_{Ax}$ values (about 1.0\times10^{-4}$ m$^2$/s) for beads of comparable density and slightly smaller diameters. It should be noted that the conductivity method used to obtain the data in figure 4 can be hindered by diffusion of calcium chloride into the beads. The time needed for 99% penetration (with $\theta_{CaCl_2} \cdot H_2O = 1.4\times10^{-9}$ m$^2$/s $\Delta t$) was calculated to be 300 s $\Delta t$, which may interfere with the dispersion measurement, causing the $D_{Ax}$ values to be slightly too high.

Axial dispersion coefficients of $10^{-3}$ to $10^{-4}$ m$^2$/s for superficial liquid velocities of 2-4$\times10^{-3}$ m/s and liquid hold ups of 50-60% the number of tanks in series by which the liquid mixing can be described is approximately 10. It was attempted to simulate the three phase FBR by sparging air into the column using various numbers of nozzles at the bottom. Initial bubble sizes were already far higher than the sizes to be expected from in-situ gas production. The sparged air eventually led to large gas bubbles clearly showing a distinct influence on the liquid flow pattern and mixing.

Figure 5: Gas hold ups in the GLR as a function of the superficial gas velocity for the gas lift reactor. The gas hold ups are in reasonable agreement with the calculated values using the hydrostatic balance, for the two phase flow system (see theory section). Furthermore, the gas hold up is seen to decrease with increasing solids hold up—this same phenomenon was reported in the literature. The relation between circulation velocity and superficial gas velocity for the GLR is depicted in figure 6. The two phase system (solids hold up=0) agrees well with the calculated values, shown as the drawn lines in figure 6. The influence of the solids hold up can be accounted for fairly well in the balance equation by using the 'corrected' volume (for solids hold up)—showing a decreasing $V_C$ value for increasing solids hold up.

Figure 6: Circulation velocities in the GLR as a function of the superficial gas velocity for various solid hold ups. (Drawn line is model calculation for two phase flow). (Drawn lines are model calculations).

Figure 7 presents the overall dispersion coefficients measured at different solid hold ups. The two series of data with zero solids hold up show that the measurements were not very accurate. Still, the two phase data conform reasonably with the values predicted by Joshi's equation (Theory). Due to this large experimental error the influence of the solids hold up on $D_{Ax}$ cannot be quantified. The axial dispersion coefficient of 2-5$\times10^{-3}$ m$^2$/s and $v_l=0.3$ m/s (at 35% solids hold up), along with a characteristic reactor length of 2.5 m leads to 100 tanks in series to represent the liquid mixing in the GLR.
Overall axial dispersion coefficients in the GLR as a function of superficial gas velocity and solids hold up. Joshi’s model equation for two phase flow (G-L) is the drawn line.

This means that the liquid mixing in one circulation of the GLR far better resembles a plug flow than one recycle in the FBR (caused by the fact that \(V\) is orders of magnitude higher in the GLR). The overall liquid mixing in the reactor is, however, determined by the recycle ratio: due to the high liquid velocities in the GLR this ratio is very high too (liquid velocities resulting from fermentation feed flow rates are approximately the same for GLR and FBR). (See also model calculations section). Visual tests (acid-base-indicator) in the GLR, operated as a three phase system, showed a plug flow behaviour in all sections of the reactor (slightly more turbulence in top section at bends) but the fast circulation (circulation time about 10 s) caused the reactor to be well mixed in a time far smaller than the liquid residence times to be expected.

MODEL CALCULATIONS

The results obtained from the hydrodynamic experiments were combined with those of the kinetic modelling\(^3,^4\) to form an integrated reactor model according to the scheme presented in figure 1. The equations of figure 1 were combined.

Equation (8) shows the explicit model equation that was used to calculate the outlet butanol concentration for both reactors, as a function of the number of tanks in series (N), the recycle ratio (\(1-\frac{L}{V}\)) and the kinetic parameters.

\[
C_b = \frac{A \cdot \left( (1 + \frac{A \cdot N}{V_b}) \right)}{((1+\frac{A \cdot N}{V_b}) - (1-\frac{L}{V_1}))}
\]

with \(A = \frac{r \cdot Y_s \cdot b \cdot s \cdot v}{N}\) (appendix)

For both reactors the outlet butanol, isopropanol and residual glucose or lactose concentration can be calculated for fermentations with Clostridium species immobilized in calcium alginate beads. For the fluidized bed reactor the desired beads fraction (solids hold up) and dilution rate overall (and substrate feed concentration) determine (all other parameters being fixed) the reactor performance. Table 1 shows the reactor performance of the 10 l FBR, expressed as the outlet butanol concentration as a function of the overall dilution rate for a certain value of \(e_s\) and substrate feed concentration.

Table 1: Reactor performance of the FBR calculated with the integrated model and compared with plug flow with recycle model and a stirred tank model (\(C_s = 35 \text{ kg/m}^3\), solids hold up 45%).

<table>
<thead>
<tr>
<th>(D_t (\text{hr}^{-1}))</th>
<th>Butanol outlet concentration (kg/m(^3))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>integrated model</td>
</tr>
<tr>
<td>N=10</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>6.53</td>
</tr>
<tr>
<td>0.3</td>
<td>5.28</td>
</tr>
<tr>
<td>0.5</td>
<td>4.43</td>
</tr>
<tr>
<td>1.0</td>
<td>3.16</td>
</tr>
<tr>
<td>1.5</td>
<td>2.46</td>
</tr>
</tbody>
</table>

This table also shows the low sensitivity of the overall reactor performance model for the hydrodynamic parameters by comparing the calculated data with those for one tank and for a plug flow with recycle. The overall FBR model best resembles that of a plug flow with recycle, but the differences between a stirred tank and a plug flow with recycle are minor.
Calculations for the GLR led to the same conclusion of low sensitivity for the hydrodynamic parameters. In part II of this study the agreement of actual fermentation data with model calculations is discussed. Table 2 shows the time constants (obtained from the hydrodynamic experiments) for axial dispersion and circulation for both reactors. They are compared with the values presented in part I of this study, which were derived from estimations and literature data. Table 2 shows a reasonable agreement between experimental values and estimated/literature values.

Table 2: Time constants-experimental values and estimations compared.

<table>
<thead>
<tr>
<th></th>
<th>Experiments</th>
<th>Literature/estimated</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBR</td>
<td>( t_{ax} = 4000 ) s</td>
<td>( t_{ax} = 20000 ) s</td>
</tr>
<tr>
<td>(solids hold up 44-45%))</td>
<td>( t_{circ} = 250 ) s</td>
<td>( t_{circ} = 200 ) s</td>
</tr>
<tr>
<td>GLR (( v_g=2.5\times10^{-2} ) m/s)</td>
<td>( t_{ax} = 1250 ) s</td>
<td>( t_{ax} = 700-1000 ) s</td>
</tr>
<tr>
<td>(solids hold up 35%)</td>
<td>( t_{circ} = 10 ) s</td>
<td>( t_{circ} = 20-40 ) s</td>
</tr>
</tbody>
</table>

CONCLUSIONS

The hydrodynamic behaviour of the 10 l. fluidized bed reactor was modelled, resulting in a 10 tanks in series model to describe liquid mixing in the reactor. The hydrodynamic parameters of the model agreed well with available literature data. No problems concerning gas release were encountered. The 15 l. gas lift loop reactor was modelled by 100 tanks in series for the liquid mixing. In accordance with literature indications gas hold ups and liquid circulation velocities were found to decrease with increasing solids hold up. This phenomenon can be described by a corrected hydrostatic balance. No significant influence of solids hold up on the overall axial dispersion coefficients were measured. Both reactor models using the developed hydrodynamic model and the already existing kinetic model were used to predict overall reactor performances. Both overall reactor models showed a low sensitivity for hydrodynamic parameters, their performances being determined by faster recirculation than conversion. Part III of the study discusses the agreement of actual fermentation data with the developed models and compares performances and operational stabilities of the two reactors, as well as the conclusions that can be drawn with respect to scale up.

REFERENCES


APPENDIX

Kinetics equations for Clostridia, immobilized in calcium alginate beads, in steady state production:

- Substrate:
  \[
  \frac{C_s - C_s^0}{(1-e)} = \frac{r_{\text{max}} (1 - C_b^m)}{C_b^m}
  \]

- Butanol:
  \[
  C_b = Y_{sb} (C_s^0 - C_s)
  \]

Isopropanol:
  \[
  C_{ip} = C_b^m / k
  \]

with:
- \(C_s^0\) = substrate feed concentration (kg/m³)
- \(C_b^m\) = substrate outlet concentration (kg/m³)
- \(r_{\text{max}}\) = maximal specific substrate consumption rate (kg/m³ alginate/hr)
- \(C_b\) = butanol outlet concentration (kg/m³)
- \(C_{ip}\) = isopropanol outlet concentration (kg/m³)
- \(Y_{sb}\) = butanol yield on substrate (kg/kg)
- \(k\) = butanol/isopropanol ratio (kg/kg)

For Clostridium species DSM 2152 on glucose at 30 °C this leads to:

\[
C_s = C_s^0 - \frac{C_s^0}{D_t} (1 - \frac{C_b}{7.4}) . 54
\]

\[
C_b = 0.22 (C_s^0 - C_s)
\]

\[
C_{ip} = C_b / 2
\]
paper VII

A comparative study of a fluidized bed reactor and a gas lift loop reactor for the IBE process.

Part III: Reactor performances and scale up.

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A COMPARATIVE STUDY OF A FLUIDIZED BED REACTOR AND A GAS LIFT LOOP REACTOR FOR THE IBE PROCESS.

Part III: Reactor performances and scale up.

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KEYWORDS: fluidized bed, gas lift loop, butanol production, scale up.

SYNOPSIS

Continuous fermentations with Clostridium species DSM 2152 immobilized in calcium alginate beads, were carried out in a fluidized bed reactor with liquid recycle (FBR, 10.9 l working volume, 41% solids) and in a gas lift loop reactor (GLR, 11.4 l working volume, 32% solids) to produce butanol and isopropanol from glucose. In the FBR a negligible influence of the in-situ produced non-coalescing gas bubbles was found on the fluidization pattern; the steady state results of the fermentation were in accordance with the model predictions, described in part II. The FBR was operated reliably for 5 weeks, without decrease of activity. The GLR clearly behaved as a three phase reactor due to the recycled fermentation gas. The steady state fermentation results were predicted within the experimental error by the GLR model. The operational stability of the reactors was studied and it was attempted to predict and compare performances of the two reactors.

INTRODUCTION

The continuous production of isopropanol-butanol or acetone-butanol mixtures by Clostridia receives increasing research interest\(^1,2\). The use of immobilized cells- more specifically the use of Clostridia immobilized in calcium alginate beads- already gave promising results\(^3,4,5\). A study of the immobilized system led to a kinetic model, applicable to various Clostridium strains and various substrates\(^6\). Lifetimes of the alginate beads with biomass were found to be long (1800 hours of continuous operation without noticeable loss of activity)\(^7\) and a combination of these immobilized cells with simultaneous recovery of the inhibiting product butanol seems attractive\(^8\). A preliminary cost estimate showed a possible economical feasibility of this process\(^9\).

For the fermentation process an analysis was made of the reactor types suitable for large scale use. In this analysis much attention was paid to the specific demands of the immobilized biocatalysts on the reactor design. Two reactor types were selected and used in the design calculations: a fluidized bed reactor with liquid recycle and an external loop gas lift reactor. Consecutively the two reactors were scaled down to laboratory scale reactors using a regime analysis based on time constants. This procedure was described in part I of this study.\(^9\) The hydrodynamics of these two reactors were modelled and combined with the kinetic equations\(^5\) to form an integrated reactor model which was described in part II.

Goal of part II of this study is to compare experimental results obtained during continuous fermentations with Cl. species, immobilized in calcium alginate beads, in the two laboratory scale reactors with the predictions obtained from the (independently developed) reactor model. Furthermore the operational stability of the reactors was studied and it was attempted to predict and compare performances of the two reactors.

MODEL EQUATIONS AND PARAMETERS

The reactor models, based on a tanks in series model, were extensively treated in part II of the study. They can be summarized by the equations (1), (2) and (3) in which the hydrodynamic aspects and kinetics of the immobilized cell system are combined to calculate the outlet substrate and product concentrations at steady state:
The parameter values for the kinetic model of immobilized Clostridium species on glucose media at 30°C are: 

\[ C_b = \frac{C_{SO} - C_s}{Y_{sb}} \times \frac{1}{v_l} \times \frac{1}{N} \]  

\[ C_{SO} = C_{SO} - C_s \]  

\[ C_{ip} = \frac{C_b}{k} \]  

with: 

- \( C_b \) = outlet substrate concentration (kg/m³) 
- \( C_{SO} \) = substrate feed concentration (kg/m³) 
- \( Y_{sb} \) = butanol yield on substrate (kg/kg) 
- \( N \) = number of tanks in series (-) 
- \( C_{BM} \) = maximum butanol concentration (kg/m³) 
- \( L \) = reactor length (m) 
- \( v_l \) = superficial liquid velocity (m/s) 
- \( D \) = total dilution rate (feed flow/total volume) 
- \( \epsilon \) = solids hold up (m³ alginate/m³) 
- \( C_{ip} \) = outlet isopropanol concentration (kg/m³) 
- \( k \) = butanol-isopropanol ratio (kg/kg)

\[ C_{SO} = C_{SO} - C_s \]

\[ C_{ip} = \frac{C_b}{k} \]  

The parameter values for the kinetic model of immobilized Clostridium species on glucose media at 30°C are:

- \( v_m \) = 54 kg glucose/m³ alginateg/hr 
- \( C_{BM} \) = 7 kg/m³ 
- \( Y_{sb} \) = 0.22 kg/kg 
- \( k \) = 2 kg/kg

The hydrodynamic parameters are:

- Fluidized bed reactor (FBR): \( N = 10, L = 2.0 \) m
- Gas lift loop reactor (GLR): \( N = 100, L = 2.5 \) m

The process variables for the reactors are \( P_c, e_c, C_{CO} \). In addition the superficial gas velocity, \( v_g \), determines \( v_l \) in the GLR; in the FBR \( v_l \) is determined by \( e_s \).

**MATERIALS AND METHODS**

**Micro-organism:** The fermentations were carried out with Clostridium species DSM 2152 (obtained from the GSF, Braunschweig, W-Germany). The cells were cultured anaerobically at 30°C in 15 ml test tubes containing media with 50 kg/m³ glucose (Baker), 10 kg/m³ yeast extract, 0.05% NaCl, and 10 kg/m³ CaCO₃. After cultivation and sporulation the micro-organisms were transferred regularly (once or twice every two weeks) to fresh media after heat shocking (1 min. 100°C, 1.5 min. 0°C). A 0.5 liter preculture (inoculated with 9th transfer tube-fermented for S-6 days) was used to inoculate a 18.0 liter fermenter (Biolafitte) containing 60 kg/m³ glucose and 10 kg/m³ yeast extract. After 6 days of fermentation at 30°C the biomass (mainly spores) was harvested by centrifugation and stored overnight at 4°C prior to immobilization. All medium components used for biomass cultivation were sterilized separately at 110°C and mixed at room temperature.

**Immobilization:** A suspension of 35 or 50 kg/m³ wet spore paste and 80 kg/m³ sodium alginate (Manucol LD, Alginate Industries) was pressed through a vibrating nozzle device, as described in part II of the study, and dropped into a 40 kg/m³ CaCl₂,2H₂O solution, thus forming the calcium alginate beads with Clostridial spores. The beads were kept overnight in the stirred solution and were then transferred to a 5 kg/m³ calcium chloride solution and stored at 4°C. (The immobilization procedure was carried out under anaerobic, non-anaerobic conditions).

**Reactors:** In part II of the study a description of the size and construction of the two reactors was given. Figures 1 and 2 are pictures taken of the fluidized bed reactor and the gas lift loop reactor respectively. Both reactors were steam sterilized (105°C, 45 min.) and all disconnections were given. Figures 3 and 4 show the arrangement of in/outlet tubes and sample port for the fluidized bed reactor. After the start-up procedure the feed flow (injected via sample in recycle loop) was initiated at the desired value; temperature control was activated (waterbath, double wall column, 30°C) and the head space of the fermenter was flushed with N₂ until the fermentation gas production was sufficiently high to maintain anaerobiosis. Part of the fermentation broth was recycled continuously, recycle ratio depending on overall dilution rate and desired fluidization velocity. The overall liquid level control consisted of a conductivity probe which activated the effluent pump. Fermentation gas was led to a gas meter and recorder to measure production rates. The FBR had a 10.9 liter working volume and a solids hold up of 42%.

**GLR:** Figure 4 schematically shows top and bottom section of the gas lift loop reactor. \( N_0 \) was initially used to circulate the reactor contents (as described in part II). When the fermentation started producing gas the \( N_0 \)-flow was decreased and eventually stopped. Part of the fermentation gas was recycled from the head space of the GLR via a peristaltic pump with gas tight tubing. Excess gas was vented and led to the gas detection device. Working volume of the GLR was 11.4 liters, solids hold up was 32%.
Figure 1: Fluidized bed reactor with liquid recycle in operation

Figure 2: Gas lift loop reactor with gas recycle in operation
Fermentation media: Media were prepared semi-continuously. Sterile tap water was obtained continuously via two prefilters (one of 0.45 x 10^-6 m and a very porous one) and a bacterial filter (0.2 x 10^-3 m) through a buffer vessel. A 40 L vessel containing solutions of glucose (500 kg/m^3) and CaCl_2*2H_2O (50 kg/m^3; technical grade) was sterilized batch-wise (60 min, 110°C) as well as a 20 L vessel with a 200 kg/m^3 yeast extract solution. From these three buffer tanks the liquids were pumped (at a certain flow rate, depending on dilution rate desired and composition) to a stirred vessel (2 L) which was cooled to 5°C. From this vessel the medium was fed to the reactor (same set up used for FBR and GLR). Medium pH was 6.0, composition: 50 kg/m^3 glucose (deliberately meant to be a large excess of substrate), 5 kg/m^3 yeast extract and 5 kg/m^3 calcium chloride. All tubings were butyl rubber, except pump tubes which were marprene or silicone, all vessels and fittings were sterilized at 110°C, 30-60 min; all medium vessels were continuously stripped with N_2.

Analyses: Liquid samples, taken at regular intervals, were centrifugated. The supernatant was used to determine butanol, isopropanol, ethanol, acetic acid, butyric acid (gas chromatographically, Chromosorb 101 column, T=145°C, 10°C/min, final hold 195°C, 2min.) and glucose (enzymatically, Boehringer hexokinase kit or YSI glucose analyser). Glucose concentrations were checked for disaccharides (on HPLC, Biorad column) which were sometimes formed due to sterilization. The residue of the samples was used for free cell biomass determinations (dry weight, 100°C, 24 hrs.). Possible infections were checked microscopically. Bead diameters were determined using stereomicroscopy. Gas production rates were calculated from observed butanol and isopropanol concentrations and the dilution rate, using the stoichiometry of the reaction equations (6 moles gas/mole isopropanol and 2 moles gas/mole butanol).

RESULTS AND DISCUSSION

In both reactors experiments were carried out to determine outlet butanol, isopropanol and glucose concentrations as a function of the dilution rate.

Model experiments. Fluidized bed reactor: After the start up procedure the reactor was operated continuously at a dilution rate (D) of 0.06 hr^-1. The liquid recycle ratio was adjusted to result in a superficial liquid velocity, v_y, of 4.2 x 10^-3 m/s; 25 hours after starting the feed flow, gas production was observed, indicating spore germination had taken place. Butanol production was observed 42 hours after start-up; the dilution rate was then increased (via 0.2 hr^-1) to 0.4 hr^-1 after which the steady state measurements were carried out. During these experiments pH was 4.5; the reactor was operated continuously for 765 hours without loss of activity. The conditions and results of the steady state experiments are summarized in table 1.
Table 1: Steady state results of FBR. (V = 10.9 l, solids hold up 41%, v = 0.0065 m/s)

<table>
<thead>
<tr>
<th>D_t (hr⁻¹)</th>
<th>Δt/τ</th>
<th>C_B (kg/m³)</th>
<th>C_IP (kg/m³)</th>
<th>ΔC_S (kg/m³)</th>
<th>ϕ_g (1/hr)</th>
<th>ϕ'_g (1/hr)</th>
<th>v_g (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.40</td>
<td>17</td>
<td>4.67</td>
<td>2.06</td>
<td>21.8</td>
<td>40</td>
<td>36</td>
<td>2.2 x 10⁻²</td>
</tr>
<tr>
<td>0.60</td>
<td>22</td>
<td>3.86</td>
<td>1.81</td>
<td>15.4</td>
<td>48</td>
<td>47</td>
<td>2.7 x 10⁻²</td>
</tr>
<tr>
<td>0.21</td>
<td>9</td>
<td>5.81</td>
<td>3.33</td>
<td>28.0</td>
<td>29</td>
<td>28</td>
<td>1.6 x 10⁻³</td>
</tr>
</tbody>
</table>

Table 1 shows the dilution rate (D_t), the number of residence times over which a steady state was measured (Δt/τ), less than 5% fluctuation in C_B, the mean butanol, isopropanol outlet concentrations (C_B, C_IP) during that steady state, the mean glucose consumption (ΔC_S, estimated from C_S₀ and C_S), the measured and calculated gas production rate (ϕ_g and ϕ'_g) and the superficial gas velocity (v_g).

Stabilization of the production after a change in dilution rate could be monitored by the gas production rate, which is directly proportional to the butanol and isopropanol production rates (proven by the measured and calculated gas production rates). Total acids concentrations (butyrate and acetate) were always below 0.55 kg/m³. Free cell biomass concentrations were below 1.0 kg/m³ in all cases. Table 2 compares the experimental results, represented by the butanol concentrations, with the values calculated with the FBR reactor model.

Table 2: Comparison experimental results with model calculations FBR.

<table>
<thead>
<tr>
<th>D_t (hr⁻¹)</th>
<th>C_B (kg/m³)</th>
<th>C_IP (kg/m³)</th>
<th>C_B (kg/m³)</th>
<th>C_B (kg/m³)</th>
<th>Experiments</th>
<th>FBR-model</th>
<th>CSTR-model</th>
<th>PF/recycle model</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.40</td>
<td>4.67</td>
<td>4.68</td>
<td>4.63</td>
<td>4.68</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.60</td>
<td>3.86</td>
<td>3.92</td>
<td>3.87</td>
<td>3.93</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0.21</td>
<td>5.81</td>
<td>5.66</td>
<td>5.61</td>
<td>5.66</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The agreement of experiments and model calculations is good; the model predictions are within the experimental error. Table 2 also shows the results of the model calculations for a CSTR and a plug flow with recycle.

Discrimination between the FBR model and plug flow with recycle model is impossible on the basis of the results presented here. The CSTR model is slightly less favourable, compared with the other two models. On laboratory scale the time constant for conversion is larger than the time constant for mixing (i.e. circulation) as was discussed in part I of the study. Therefore the difference between the CSTR model and the plug flow model was small. The dispersion refinement (N-tanks in series with recycle or plug flow with recycle) cannot be noticed at all on laboratory scale.

Gas lift loop reactor: After starting up the dilution rate was set at 0.09 hr⁻¹. When gas production and microbial activity were observed (24 hrs, after starting) the D_t was increased gradually to 0.30 hr⁻¹, after which the steady state measurements began. Fermentation pH was 4.4. Table 3 shows the results of these steady state experiments, in the same way as was explained for the FBR.

Table 3: Steady state results of GLR(V = 11.4 l, solids hold up 32%, v = 0.025 m/s)

<table>
<thead>
<tr>
<th>D_t (hr⁻¹)</th>
<th>Δt/τ</th>
<th>C_B (kg/m³)</th>
<th>C_IP (kg/m³)</th>
<th>ΔC_S (kg/m³)</th>
<th>ϕ_g (1/hr)</th>
<th>ϕ'_g (1/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.30</td>
<td>12</td>
<td>4.61</td>
<td>2.64</td>
<td>20.4</td>
<td>22.5</td>
<td>31.5</td>
</tr>
<tr>
<td>0.44</td>
<td>12</td>
<td>4.09</td>
<td>2.15</td>
<td>17.3</td>
<td>30.0</td>
<td>38.9</td>
</tr>
<tr>
<td>0.58</td>
<td>8</td>
<td>3.42</td>
<td>1.74</td>
<td>15.0</td>
<td>36.0</td>
<td>42.5</td>
</tr>
<tr>
<td>0.19</td>
<td>20</td>
<td>5.37</td>
<td>2.97</td>
<td>23.6</td>
<td>20.0</td>
<td>23.5</td>
</tr>
</tbody>
</table>

The measured and calculated gas production rates show a systematic discrepancy which may have been caused by a gas leak in the recycle loop. Gas hold ups during the experiments were approximately 2.5%. The GLR was operated continuously for 640 hrs without noticeable loss of activity. Table 4 shows the experimental and model C_B values. There is good agreement between experiments and models, no discrimination is possible between the actual GLR model (100 tanks in series) and the other two models. The close resemblance between the CSTR model and the plug flow model is entirely due to the short circulation times (i.e. mixing times) in comparison with the time constant for production, causing the reactor to be well mixed.
Table 4: Comparison of experimental results and model predictions in the GLR.

<table>
<thead>
<tr>
<th>$D_t$ (hr$^{-1}$)</th>
<th>$C_B$ (kg/m$^3$)</th>
<th>$C_B$ (kg/m$^3$)</th>
<th>$C_B$ (kg/m$^3$)</th>
<th>$C_B$ (kg/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiments</td>
<td>GLR-model</td>
<td>CSTR-model</td>
<td>PF/recycle-model</td>
</tr>
<tr>
<td>0.30</td>
<td>4.61</td>
<td>4.67</td>
<td>4.67</td>
<td>4.67</td>
</tr>
<tr>
<td>0.44</td>
<td>4.09</td>
<td>3.98</td>
<td>3.98</td>
<td>3.98</td>
</tr>
<tr>
<td>0.58</td>
<td>3.42</td>
<td>3.45</td>
<td>3.45</td>
<td>3.45</td>
</tr>
<tr>
<td>0.19</td>
<td>5.37</td>
<td>5.34</td>
<td>5.34</td>
<td>5.34</td>
</tr>
</tbody>
</table>

Operational aspects.

Fluidized bed reactor: Figure 5 shows a picture of the alginate beads in the FBR during the actual fermentation. The picture was taken near the top of the column. The beads (1.9x10$^{-3}$ m diameter) are clearly larger than the gas bubbles. The gas bubbles were immediately released after formation, did not cling to the beads and did not visibly change in diameter over the length of the column, indicating there was hardly any coalescence. This is in agreement with literature data, indicating small bubbles in low Reynolds number regions behave as rigid spheres and do not coalesce. Furthermore, the gas moved regularly through the bed without wall effects and without use of preferential pathways. The fluidization pattern of the alginate beads was very regular, seemingly undisturbed by the gas bubbles. The bed expansion was not visibly influenced by the gas phase.

Foam formation did not occur, apart from a little during the first 24 hours of the fermentation. (Whey permeate medium was tested specifically for foaming—did not lead to foaming either).

The reactor was exposed to oxygen shock load, leading to a 3 ppm $O_2$ concentration in the effluent. This caused the immobilized Clostridia to rapidly cease fermentation (gas production rates dropped). However, when the dissolved oxygen concentration dropped just below 1 ppm fermentation activity rapidly increased and gas production rates were back to "normal". This shows that the obligate anaerobic Clostridia are not extremely oxygen sensitive when immobilized.

Some tests were taken by deliberately opening the reactor and stirring the contents with a contaminated bar. No effect of this was observed and the production continued as normal.
Bead attrition rates were found to be below detection after the 30 days of continuous operation.

Stopping of the liquid recycle flow causes the beads to settle (fluidization stops) and form a packed bed. Immediately gas plugs, consisting of accumulated, non-coalesced bubbles form and, stabilized by the reactor walls, these plugs lift the bed. This shows the impossibility to operate this fermentation with immobilized cells as a packed bed process.

Concludingly it can be stated that for five weeks the 10 l. FBR showed a steady reactor behaviour with regular fluidization and little effect of the gas phase; no decrease in activity or productivity was observed.

Gas lift loop reactor: During the continuous experiments in the GLR the same low infection sensitiveness as for the FBR was found. Due to the fact that gas was sparged into the reactor foam formation did occur in the reactor, causing the level control system to malfunction. Foaming could be suppressed by antifoaming agent (P2000), but this caused increased coalescence of the gas bubbles and diminished gas hold ups; this resulted in a temporary unsteady circulation of the reactor contents. Apart from this the laboratory scale GLR was found to operate satisfactorily. Recycling of the fermentation gas was not problematic; bead attrition rates were again below detection.

Scale up.

In part I of this study it was described how the laboratory scale FBR and GLR were designed as models of two full scale reactors. This scale down procedure was carried out with a regime analysis, using the time constants of the important processes in the reactors. These time constants were obtained from literature data or estimated. Part II shows the composition of mathematical models, combining kinetic and hydrodynamic models, to predict reactor performances. In the underlying part II the results of these models are compared with experimental results. For each reactor it is now discussed which conclusions can be drawn with respect to scale up, after which the reactors will be compared.

Fluidized bed reactor: A distinction is made between the validity of the laboratory scale experiments to draw conclusions with respect to the hydrodynamic behaviour of a 50 m³ FBR and the validity of these experiments to predict reactor performances on 50 m³ scale.

Of importance to comment on the full scale hydrodynamic behaviour is the gas phase in the FBR. The laboratory scale FBR behaved seemingly as a liquid fluidized bed, with no influence of the in-situ produced gas bubbles on the bed expansion and a regular fluidization pattern.

For the 50 m³ FBR three points are important to describe the gas phase behaviour in this reactor (H/D=3, D=2.8 m)

1. Superficial gas velocities (calculated from model equations and stoichiometry of the reaction) in the FBR will approximately be 4×10⁻³ – 6×10⁻³ m/s. This results in gas hold ups of 1-2% (calculated from sup. gas velocity and bubble rise velocity). In the lab.scale FBR gas velocities of 1-3×10⁻³ m/s were found, which is 50-75% of the full scale value.

2. Pressure effects in the full scale FBR (H=8.3 m) are unlikely to lead to gas bubbles much differing in diameter from the ones observed on laboratory scale (0.5-1.0×10⁻⁰ m)

3. Extremely high gas hold ups (10-15%) and superficial gas velocities (60×10⁻³ m/s) can be handled by the laboratory scale reactor (this was tested in part II).

On the basis of these three points the behaviour of the gas phase observed on laboratory scale was thought to be representative for and translatable to the production scale situation. For the liquid and solid phase behaviour on full scale, it is of importance to construct a bottom plate which guarantees even liquid distribution to prevent radial gradients and ensure regular fluidization of the biocatalyst particles.

Packing of the bed on production scale, as was observed on laboratory scale is unlikely. If the liquid recycle should be disturbed (to be avoided of course) and the reactor bed settles, there is little chance gas plugs of the size of the column diameter will be formed. The gas is likely to escape via preferential pathways and break through the bed.

The validity of the laboratory scale experiments to predict reactor performances on full scale is thought to be large.
Table 5 shows the results of the model calculations for the 50 m$^3$ FBR. The number of tanks in series (calculated from $v \cdot L / 2 \cdot D_{\text{ax}}$, see part II) was determined using a $v_1$ of 5*10$^{-3}$ m/s, solids hold up of 45% and $D_{\text{ax}} = 1.0 \times 10^{-3}$ m$^2$/s (values directly obtained from hydrodynamic experiments in part II); this leads to a 38 tanks in series model, which is virtually plug flow, to represent the liquid mixing in one loop through the 50 m$^3$ fluidized bed reactor. The simple plug flow with recycle model is therefore preferred to describe the reactor performance on production scale.

The fact that the conversion time on production scale is larger than the circulation (mixing) time leads to the small influence of mixing on the reactor performance on production scale. The fact that the circulation time on production scale is larger than the circulation time on laboratory scale leads to a decreased difference between production/conversion time (equal on both scales) and circulation time, through which the influence of mixing is somewhat larger on production scale than on laboratory scale (expressed as a larger difference between PF/recycle prediction and CSTR prediction). The relatively large production time is, as already said, the cause of this insensitivity for mixing on the performance. The strain used, Cl. species, has a reasonably high productivity, but other strains are being reported\textsuperscript{3,13} (sometimes mutant strains) with high production rates and high butanol tolerancies. These strains, when immobilized, could well lead to a time constant for production/conversion 3-5 times lower than found for Cl. species (see Appendix), thus diminishing the difference between mixing time and production time and increasing the difference in performance (and outlet butanol concentration) between the FBR described with the plug flow with recycle model and the FBR described with a CSTR model.

Table 5: Model calculations for the 50 m$^3$ FBR.

<table>
<thead>
<tr>
<th>$D_1$ (hr$^{-1}$)</th>
<th>$C_B$ (kg/m$^3$)</th>
<th>$C_B$ (kg/m$^3$)</th>
<th>$C_B$ (kg/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FBR-model</td>
<td>PF/recycle-model</td>
<td>CSTR-model</td>
</tr>
<tr>
<td>0.1</td>
<td>6.62</td>
<td>6.62</td>
<td>6.50</td>
</tr>
<tr>
<td>0.3</td>
<td>5.46</td>
<td>5.46</td>
<td>5.23</td>
</tr>
<tr>
<td>0.5</td>
<td>4.64</td>
<td>4.64</td>
<td>4.37</td>
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<tr>
<td>1.0</td>
<td>3.38</td>
<td>3.38</td>
<td>3.10</td>
</tr>
<tr>
<td>1.5</td>
<td>2.66</td>
<td>2.66</td>
<td>2.41</td>
</tr>
</tbody>
</table>

The fact that the conversion time on production scale is larger than the circulation (mixing) time leads to the small influence of mixing on the reactor performance on production scale. The fact that the circulation time on production scale is larger than the circulation time on laboratory scale leads to a decreased difference between production/conversion time (equal on both scales) and circulation time, through which the influence of mixing is somewhat larger on production scale than on laboratory scale (expressed as a larger difference between PF/recycle prediction and CSTR prediction).

More difficult to predict from the laboratory scale data is the hydrodynamic behaviour of the 65 m$^3$ GLR. Table 7 shows the calculated circulation velocities and gas hold ups as a function of the superficial gas velocities for the full scale GLR. The hydrostatic balances, corrected for the three phase situation, as described in part II for the laboratory scale GLR were used. The axial dispersion coefficient, $D_{\text{ax}}$, was calculated using Joshi's equation (see part II). Table 7 furthermore shows the resulting number of tanks in series (obtained from $v \cdot L / 2 \cdot D_{\text{ax}}$) to describe the liquid mixing in one recycle loop in the GLR.

Table 6: Outlet butanol concentrations for the 65 m$^3$ GLR, operated at $D_1 = 0.3$ hr$^{-1}$.

<table>
<thead>
<tr>
<th>$v_g$ (m/s)</th>
<th>$C_B$ (kg/m$^3$)</th>
<th>$C_B$ (kg/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSTR-model</td>
<td>PF/recycle-model</td>
</tr>
<tr>
<td>0.01</td>
<td>4.824</td>
<td>4.829</td>
</tr>
<tr>
<td>0.03</td>
<td>4.824</td>
<td>4.827</td>
</tr>
<tr>
<td>0.05</td>
<td>4.824</td>
<td>4.827</td>
</tr>
</tbody>
</table>

Of course, it must be borne in mind that too large a gradient over the fluidized bed is unfavourable for the immobilized biomass (part I), therefore these considerations will always lead to a compromise (or optimum) between production time and mixing time.

Gas Lift Loop Reactor: In part I of the study the full scale GLR was designed to contain similar amounts of biocatalyst particles, leading to a reactor of 65 m$^3$, solids hold up is 35% ($H/D = 5$, $D_r = D_d = 2.0$ m). Conclusions have to be drawn with respect to performance and hydrodynamic behaviour on 65 m$^3$ scale in the GLR. The large scale performance is predicted by a CSTR model as can be seen in Table 6. The reactor will be well mixed on a large scale, similar to the situation on laboratory scale (with liquid moving as a plug through the reactor, at high circulation rates), mixing times are far smaller than conversion times.

Table 7: Outlet butanol concentrations for the 65 m$^3$ GLR, operated at $D_1 = 0.3$ hr$^{-1}$.

<table>
<thead>
<tr>
<th>$v_1$ (m/s)</th>
<th>$C_B$ (kg/m$^3$)</th>
<th>$C_B$ (kg/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSTR-model</td>
<td>PF/recycle-model</td>
</tr>
<tr>
<td>0.01</td>
<td>4.824</td>
<td>4.829</td>
</tr>
<tr>
<td>0.03</td>
<td>4.824</td>
<td>4.827</td>
</tr>
<tr>
<td>0.05</td>
<td>4.824</td>
<td>4.827</td>
</tr>
</tbody>
</table>
On production scale, as on laboratory scale, one loop through the reactor will have a plug flow character. The fast circulation times, compared with the conversion times, cause the reactor to be well mixed and this is unlikely to change when the conversion times would decrease with a factor \(3\rightarrow 4\) (circulation still much faster than conversion).

As was already stated in part I, the validity of the extrapolations from laboratory scale for the gas phase behaviour in the full scale GLR is less certain than the predictions presented here.

**Table 7**: Hydrodynamic parameters in the 65 \(m^3\) GLR, as a function of the superficial gas velocity, calculated with hydrostatic balances. \((L=20.2\ m\), solids hold up is 35%; \(v_c\) is circulation velocity, \(D_{ax}\) is axial dispersion coefficient, \(N\) is number of tanks).

<table>
<thead>
<tr>
<th>(v_g) (m/s)</th>
<th>(e_g) (%)</th>
<th>(v_c) (m/s)</th>
<th>(D_{ax}) (m(^2)/s)</th>
<th>(N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>0.68</td>
<td>0.53</td>
<td>0.39</td>
<td>14</td>
</tr>
<tr>
<td>0.03</td>
<td>1.49</td>
<td>0.79</td>
<td>0.56</td>
<td>14</td>
</tr>
<tr>
<td>0.05</td>
<td>2.13</td>
<td>0.94</td>
<td>0.67</td>
<td>14</td>
</tr>
</tbody>
</table>

In part II of the study no mechanistic description of the gas phase behaviour in the complex three phase GLR was given, although a method was used to calculate gas hold ups and circulation velocities as a function of the (free to choose) superficial gas velocity and the solids hold up. The gas hold ups were found to decrease with increasing solids hold up, which is not likely to be scale dependent. However, other effects (e.g. pressure effects on full scale, wall effects possibly present on laboratory scale, different coalescing behaviour on full scale) may cause the results in table 7 to give a less realistic description of the gas hold up on production scale and should therefore be interpreted with caution. Apart from the uncertainty in the gas hold up predictions, the hydrostatic balance method (part II) with friction losses calculated for 65 \(m^3\) scale seems useful to predict circulation velocities in the three phase GLR.

Figure 6: Reactor productivity and substrate consumption (\(\Delta C_s\)) as a function of the feed flow rate (\(\phi_{in}\)) for the 50 \(m^3\) FBR and the 65 \(m^3\) GLR.
**FBR-GLR comparison** The aspects of importance for a large scale applicability of either the fluidized bed reactor or the external loop gas lift reactor in the IBE process are summarized in Table 8.

Table 8: Comparison of 50 m$^3$ FBR and 65 m$^3$ GLR.

<table>
<thead>
<tr>
<th></th>
<th>FBR</th>
<th>GLR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactor productivity</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Biocatalyst attrition rates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reactor construction</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Construction bottom plate</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ease of operation</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Foaming</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Gas release</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Combination simultaneous</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Recovery process</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

(+ is favourable; - is unfavourable or attention needed)

Note: * based on results presented here and assumptions made in part I.

The first item in Table 8, reactor performance, is further elucidated in Figure 6. For a given feed flow rate $\dot{V}_{\text{in}}$ and a substrate feed concentration of 35 kg/m$^3$ the reactor productivity (obtained from (butanol+isopropanol concentration)*dilution rate), presented for both the 50 m$^3$ FBR and the 65 m$^3$ GLR, was calculated using the plug flow with recycle model for the FBR and the stirred tank model for the GLR. The reactors were designed (in part I) to contain equal amounts of biocatalyst (45% in the 50 m$^3$ FBR and 35% in the 65 m$^3$ GLR) and to produce equal annual amounts of butanol-isopropanol. This automatically leads to a higher productivity for the FBR. Furthermore, the plug flow with recycle character of the 50 m$^3$ FBR leads to slightly higher product concentrations than the 65 m$^3$ GLR with its stirred tank character, thus increasing the FBR’s productivity even more. As was already mentioned when discussing the FBR, this difference in performance would become more pronounced for Clostridium strains with higher production rates, i.e., lower time constant for production/conversion.

One aspect of Table 8 deserves further comment: integration with the product recovery process. As was already discussed in the introduction, simultaneous product recovery can increase the reactor productivity. The large scale FBR allows this integration without much effort via its recycle loop. The biocatalyst beads will not be in contact with the recovery section and will only ‘view’ the treated and recycled fermentation broth. For the GLR the recovery section will either be situated in the reactor, which is not easy from a construction/operation point of view, or an extra liquid loop will have to be used to draw the broth from the GLR, treat it and recycle it (energy consumption!)

Table 8 shows the fluidized bed with liquid recycle as a better option than the 65 m$^3$ gas lift loop reactor for application in the large scale butanol/isopropanol process with immobilized Clostridia.

**CONCLUSIONS**

**Fluidized bed reactor:** Butanol outlet concentrations at steady state are predicted within the experimental error by the FBR model, based on two phase fluidization and 10 tanks in series to describe the liquid mixing in the bed. In-situ produced fermentation gas bubbles are small (0.5-1.0*10$^{-3}$ m) and behave as rigid spheres. For a three phase reactor with a gas gradient over the reactor bed the FBR showed a remarkably steady, regular fluidization and reliable operation, with no decrease in activity.

**Gas lift loop reactor:** The three phase reactor model for the GLR, based on plug flow and fast circulation, predicts the outlet butanol concentration well as a function of the dilution rate; influence of the hydrodynamic parameters is small. Recycling part of the fermentation gas for liquid mixing was not problematic; the GLR ran steadily for four weeks without decrease in activity.

For both reactors bead attrition rates were found to be below detection.

**Scale-up:** Performances of the two reactors on 50 m$^3$ (FBR) and 65 m$^3$ (GLR) scale were thought to be predictable by the models: a plug flow with recycle model for the 50 m$^3$ FBR and a stirred tank model for the 65 m$^3$ GLR. Hydrodynamic behaviour of the 50 m$^3$ FBR was considered not to differ substantially from laboratory scale: regular fluidization, in-situ produced gas bubbles of appr. 0.5-1.0*10$^{-3}$ m, superficial gas velocities of 1.3-2.0 times the value on laboratory scale.
The GLR (65 m³) gas phase behavior is more difficult to predict from the laboratory scale data.

Comparison of the FBR and GLR leads to a 22% higher production rate per unit reactor volume for the FBR (due to higher solids hold up), a slightly higher outlet product concentration for the FBR (due to its plug flow with recycle character) which will become more pronounced when using improved strains with higher production rates. Combining this with ease of integration of the FBR with a simultaneous product recovery leads to a preference of the FBR for application in the butanol/isopropanol process with immobilized Clostridia.

REFERENCES


APPENDIX

The influence of the kinetic parameters (i.e. specific of immobilized strain) on the time constants for conversion/production and circulation is expressed in table 1a. Starting from the base case for the 50 m³ fluidized bed reactor (for immobilized Cl. species) the influence of the maximal butanol concentration, C BM, was examined (C BM = 7.4 kg/m³ for immobilized Cl. species). Furthermore the influence of increased r max values was included; r max is a kinetic parameter expressing the maximal substrate consumption rate of the immobilized microorganism (r max = 54 kg glucose/m³ alginate/hr for Cl. species). Increase of r max can be achieved by increasing the concentration of immobilized cells.

Table 1a. Time constants for improved strains.

<table>
<thead>
<tr>
<th>CBM (kg/m³)</th>
<th>r max (kg/m³ alg.hr)</th>
<th>t conversion (s)</th>
<th>t circulation (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.4</td>
<td>54</td>
<td>-1.2 x 10⁴</td>
<td>7.5 x 10⁴</td>
</tr>
<tr>
<td>15</td>
<td>54</td>
<td>-3.6 x 10³</td>
<td>8.5 x 10³</td>
</tr>
<tr>
<td>20</td>
<td>110</td>
<td>-2.4 x 10³</td>
<td>1.8 x 10³</td>
</tr>
<tr>
<td>150</td>
<td>20</td>
<td>-2.0 x 10³</td>
<td>2.7 x 10³</td>
</tr>
</tbody>
</table>

The time constants are calculated according to the methods described in part 19 and aimed at the same outlet butanol concentration for the 50 m³ FBR; this means there is less time required for the strain with the highest values of CBM and r max to reach the desired butanol outlet concentration in the 50 m³ FBR.

Improved strains of course lead to an improved performance of the FBR, but also to an increased difference in performance between the FBR, described with a plug flow/recycle model and with a CSTR model. Over the data given in table 1a the total reactor productivity increases with a factor 1 to 4.5, the relative difference between a plug flow with recycle and a CSTR increases with a factor 1 to 9.5. This indicates the enhanced preference for a fluidized bed reactor with liquid recycle when more productive strains are used.
Economic feasibility of the production of isopropanol-butanol-ethanol fuels from whey permeate.

ECONOMIC FEASIBILITY OF THE PRODUCTION OF ISO-PROPANOL–BUTANOL–ETHANOL FUELS FROM WHEY PERMEATE

By G.H. SCHOUTENS and W.J. GROOT

Laboratory of Bioengineering, Department of Chemical Engineering, Delft University of Technology, Julianalaan 67, 2628 BC, Delft, The Netherlands.

Introduction

In 1980 a cost calculation of a butanol-ethanol production plant was published. The calculation attempted to compare the costs of ethanol, aceton, and butanol produced fermentatively from whey with the costs of the same products produced from oil; the price of oil had been rising from 1973 on. The plant described operated as a traditional batch process and the products were recovered by conventional distillation.

In this paper an estimation is made of the production costs of fermentatively produced iso-propanol–butanol–ethanol mixtures produced as a by-product of the same annual production as that of the plant cited, i.e. 45 × 10^6 kg IBE year–¹ but operated in an entirely different way. The three main differences are:

1. The reactors are operated continuously.
2. The fermentation is carried out by immobilized micro-organisms.
3. The products are recovered continuously and simultaneously by means of pervaporation, thus increasing the substrate conversion rate.

The aim of this study was to calculate whether use of these micro-organisms can ferment lactose-containing substrates to mixtures of iso-prop?nol. butanol. and ethanol...
99.5% IBE mixture, at the 1984 price-level. The costs are... from investments. The combination of a concentrated feed (low transport costs) and by the salts concentration of the pervaporate at condensation temperature. In practice, however, leakage in the system will necessitate to maintain vacuum by vacuum pumping. It was assumed that this will cost 10% of the energy involved with the vaporization of the liquids, supplied as electricity.

The table below shows the influence of this distance on the total production costs.}

Table 2: Influence of the Whey Permeate Transport Distance on the Total Product Price

<table>
<thead>
<tr>
<th>Distance (km)</th>
<th>Product price (US$ kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.149</td>
</tr>
<tr>
<td>5</td>
<td>0.168</td>
</tr>
<tr>
<td>100</td>
<td>0.188</td>
</tr>
<tr>
<td>150</td>
<td>0.207</td>
</tr>
<tr>
<td>200</td>
<td>0.226</td>
</tr>
<tr>
<td>250</td>
<td>0.245</td>
</tr>
<tr>
<td>300</td>
<td>0.264</td>
</tr>
<tr>
<td>400</td>
<td>0.283</td>
</tr>
<tr>
<td>500</td>
<td>0.302</td>
</tr>
<tr>
<td>600</td>
<td>0.321</td>
</tr>
<tr>
<td>700</td>
<td>0.340</td>
</tr>
</tbody>
</table>

Discussion and Conclusions

This paper describes the framework within which the IBE mixture can be produced at a price comparable with or lower than that of gasoline. The resulting cost is still preliminary, based partly on experimental data, partly on theoretical assumptions, but it nevertheless shows that the IBE process is economically feasible.

Although some information is available already, the contributions characteristics and yields of the IBE mixture have to be studied more closely to be able to compare it in greater detail with gasoline. This comparison will then be able to determine the ultimate IBE price at which the process is still competitive with gasoline, which will lead to the maximum price level of the bulk whey permeate.

Furthermore, it has been shown that the use of relatively new technologies like immobilization of cells and membrane processes for product recovery through the high production rates they accommodate can be feasible at a large scale.

The combination of a concentrated feed (low transport costs), immobilized cells, and continuous product removal will reduce the investment costs of the plant significantly, thereby increasing the economic attractiveness of the IBE process.

Table 3: Influence of the Biocatalyst Renewal Frequency on the Total Product Price

<table>
<thead>
<tr>
<th>Renewal frequency (times per year)</th>
<th>Product price (US$ kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-case</td>
<td>0.149</td>
</tr>
<tr>
<td>Case</td>
<td>0.126</td>
</tr>
</tbody>
</table>
SCHOUTENS and GROOT. ECONOMIC FEASIBILITY OF PRODUCTION OF FUELS FROM WHEY

(high reaction rates) is essential. However, this preliminary design is not optimized yet.

Factors that could further increase the profit margin are:

(1): Further purification of the IBE mixture will lead to products (n-butanol, iso-propanol and ethanol) with a far higher market value than the fuel-mixture.

(2): Use of the IBE mixture as an additive for unleaded gasoline to increase the octane number. (This IBE mixture is miscible with gasoline in any ratio.)

(3): Collection and further processing of the fermentation gas (CO₂ and H₂); use of the waste from the beer still for methane production.

(4): Use of a reverse osmosis process instead of an evaporation process for the concentration of the whey permeate. Such a process would probably be cheaper and easy to install behind an ultrafiltration unit.

(5): Lowering of the salts concentration in the whey permeate; feed concentrations higher than 27.5% (w/v) could then be used, which would lead to lower product recovery costs.

(6): Optimization of the energy balance of the recovery section.

(7): Situation of the fermentation plant next to the whey permeate producing factory; the production costs would be 20% lower.

Acknowledgement

The authors wish to thank Dr. Ir. N. W. F. Kossen for his advice.

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APPLICATION OF ISOPROPANOL-BUTANOL-ETHANOL MIXTURES AS AN ENGINE FUEL

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The application of fermentatively produced alcohols as engine fuels receives increasing attention. The combination of traditional fermentation know-how with new technologies, a low cost raw material and an attractive product (like a fuel) can become profitable. In France and Japan research efforts in the area of ethanol and acetone-butanol production by cellulytic fermentations already resulted in plans for pilot plants and production plants. The use of alcohols as octane boosters in unleaded gasoline is rapidly becoming popular and will also have its effect on the applicability and market value of fermentatively produced alcohols.

A recent preliminary costing study on the continuous production of isopropanol-butanol-ethanol mixtures (IBE) from fermentation of whey permeate (dairy by-product) shows that this alcohol mixture can be produced at a price competitive with that of gasoline, provided certain boundary conditions are met.

Engine performance experiments were carried out with this particular isopropanol-butanol-ethanol mixture in order to be able to compare the production price of this mixture more thoroughly with that of ordinary gasoline. This price comparison is of importance to get a realistic view of the applicability of this alcohol mixture as a neat engine fuel.

A 1.9 liter Opel Manta test engine with electronic fuel injection was used to test the mixture, which consisted of butanol, isopropanol, ethanol (on a 6:3:1 weight ratio) and water (0.5%). Ordinary gasoline (super) was used as a blank test medium.

The experiments were carried out by monitoring the fuel mass flow (g/s), emission levels of CO, CO₂ and O₂ (v/v), NOₓ and CH-residues (ppm v/v) and several other engine characteristics (air/fuel ratio, temperatures, pressures) as a function of the engine load, expressed as brake mean eff. pressure (bmepe(bar)), and the number of revolutions (min⁻¹). From these data the brake specific fuel consumption (g/kWh) was calculated for gasoline and the IBE mixture.

Instability of the engine or discontinuities during the runs on the IBE—mixture did not occur. Due to the fact that the experiments were carried out in an engine with fuel injection, no problems concerning adjustment of the air/fuel ratio were encountered.

In table 1 the most important results of the experiments are summarized. In this table the differences between the IBE mixture and gasoline are shown.

Table 1: Results of the engine performance test on IBE, compared with those on gasoline.

<table>
<thead>
<tr>
<th>Revolutions</th>
<th>Load</th>
<th>Relative fuel mass flow</th>
<th>Relative spec. fuel consumption</th>
<th>Relative NOₓ emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>low</td>
<td>low</td>
<td>+ 25%</td>
<td>+ 20%</td>
<td>- 55%</td>
</tr>
<tr>
<td>low</td>
<td>medium</td>
<td>+ 18%</td>
<td>+ 20%</td>
<td>- 21%</td>
</tr>
<tr>
<td>low</td>
<td>max</td>
<td>+ 27%</td>
<td>+ 27%</td>
<td>- 20%</td>
</tr>
<tr>
<td>high</td>
<td>low</td>
<td>+ 16%</td>
<td>+ 22%</td>
<td>- 44%</td>
</tr>
<tr>
<td>high</td>
<td>medium</td>
<td>+ 21%</td>
<td>+ 20%</td>
<td>- 24%</td>
</tr>
<tr>
<td>high</td>
<td>max</td>
<td>+ 24%</td>
<td>+ 26%</td>
<td>- 14%</td>
</tr>
</tbody>
</table>

On average the specific fuel consumption on the IBE mixture was 22.5% higher than on gasoline, the NOₓ emission was about 30% lower. Furthermore, the average emission levels of CO and CH-residues were comparable for both fuels.

In the costing paper the price of the IBE mixture was estimated to be about US $0.19/kg; that of gasoline (production price, 1984) was estimated to be US $0.30-0.50/kg. As the specific fuel consumption for IBE is 22.5% higher, the IBE price is more realistically approached by US $0.19 + $0.225 x 0.19 = US $0.23/kg gasoline equivalent.
This means that accounting for the lower engine performance on IBE, the realistic IBE fuel price still is comparable with that of gasoline, when it is produced under the conditions indicated in the costing study. No experiments were carried out to test the octane boosting capacity of IBE in unleaded gasoline. However, from literature data it can be estimated that the research octane number (RON) will be somewhere in between that of methanol and methyl tertiary butyl ether (MTBE). In certain types of unleaded gasoline a mixture of 2% methanol and 2% MTBE is (amongst others) used as additive. The RON number of IBE will be comparable to that of the 2% MeOH/MTBE mixture. The price of this mixture is about US $0.25-0.30/kg (about US $0.18/kg MeOH and US $0.38/kg MTBE) which means that the production price of IBE under the conditions in the costing paper is comparable. From these rough estimations and the fact that IBE does not cause any obvious engine problems and is miscible in nearly any ratio with gasoline it can be concluded that the application of IBE as a fuel additive to unleaded gasoline certainly looks promising. The IBE process may become important as it combines an attractive product with a flexible and robust fermentation process. It can use a wide variety of carbohydrates as raw material, which allows the process to be tuned to the local raw material availability and, more important, the application of the product IBE in that same area.

References:

Curriculum vitae.

Permission was granted for all reprints.

ERRATA:

Paper I: Summary, first line: "LMD 27.7" should be "LMD 27.6".

Paper VIII: Figure 2, stream 2: "16600" should be "166000".

STELLINGEN

behorende bij het proefschrift
"Modelling and design of an immobilized cell process for solvent production"

1. In anaerobie is obligaat een relatief begrip dat nadere specificatie verlangt.

2. Het gebruik van Manucol LD alginaat voor immobilisatie van Clostridia maakt het, in de literatuur als essentieel voor een lange levensduur gepropageerde, drogen van alginaatdeeltjes ter versteviging van de matrix overbodig.

3. De tienvoudige toename in butanolproductiviteit in een geïmmobiliseerd celsysteem, die door Leung en Wang in de samenvatting van een publikatie over vrije celsystemen wordt gedaan, berust op een vergissing.

4. Het vergelijken van gemiddelde reaktorproductiviteiten uit diverse bronnen is ongeoorlofend en nietszeggend als de bronnen verschillende definities van dit begrip hanteren.

5. In het technologisch jargon is "k_a" tot een op zichzelf staand begrip verheven, waarbij de oorsprong ervan - te weten het produkt van een overdrachtscoëfficiënt en een specifiek oppervlak - (te) vaak uit het oog verloren wordt.

6. Het "educated incapacity syndrome" dat zou heersen in de hedendaagse biotechnologie, heeft veel minder betrekking op de koppeling tussen fundamenteel en toegepast dan wel op de koppeling tussen bio en technologie.


7. Het "brain drain"-effect kan wellicht worden tegengegaan door een "brain retain"-effect, te bewerkstelligen door het invoeren van sabbatical years voor wetenschappelijke stafleden.

8. Een "brain gain"-effect kan bereikt worden door promovendi buitenlandse studiereizen in een "brain train"-programma aan te bieden.

9. Te oordelen naar het aanbod van patiënten op een EHBO-post kan men de levensverwachting aanzienlijk verlengen door niet te sporten, geen huisdieren te houden en niet op een brommer te rijden.

15 mei 1986
Gerda Schoutens