Continuous Pilot Plant–Scale Immobilization of Yeast in κ-Carrageenan Gel Beads

C. Decamps and S. Norton
Labatt Breweries of Canada, PB 5050, London N6A 4M3, Canada

D. Poncelet
Ecole Nationale d'Ingénieurs des Techniques des Industries Agricoles at Alimentaires (ENITIAA), BP 82225, 44322 Nantes Cedex 3, France

R. J. Neufeld
Dept. of Chemical Engineering, Queen's University, Kingston K7L 3N6, Canada

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A novel continuous two-phase dispersion process was developed to produce κ-carrageenan gel microspheres, using static mixers. It was shown that yeast-loaded carrageenan beads, with controlled diameter and tight size distribution, can be produced on a continuous basis, in a scalable mixer, at production rates appropriate to both pilot plant–scale and, potentially, industrial-scale operations. Immobilized yeast are intended to be used in continuous brewing operations. The effects of the static mixer diameter (D), the number of mixing elements (N_e), the fluid linear velocity (V), and the volumetric fraction (ϵ) of κ-carrageenan, on the mean diameter and size distribution of the resulting gel microspheres, were studied. Image analysis showed that mean diameter was strongly influenced by the average linear fluid velocity through the mixer, and by the mixer diameter. The number of mixer elements and the mixer diameter governed bead size dispersion. A productivity of 10 L h^{-1} of beads was attained using a 1.27-cm-diameter static mixer. Because the productivity is proportional to the mixer diameter squared, this process, although suited for the production of small-size beads (down to 50 μm), would be technically and economically feasible for a large industrial immobilization process. However, because the coefficient of variability increased with mixer diameter, and thus with scale-up, operational improvements are suggested, such as the use of smaller-diameter mixers operating in parallel, to reduce the size dispersion. © 2004 American Institute of Chemical Engineers *AIChE J,* 50: 1599–1605, 2004
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Introduction

The overall objective of this study was to design, construct, and optimize a continuous process for the production of yeast-inoculated gel beads at a pilot scale and to produce beer using immobilized cells in a continuous gaslift bioreactor. Lager beer requires a primary batch fermentation time of 6–7 days; thus there is international interest in developing a more economical, smaller-scale, continuous fermentation process using immobilized cells for the primary fermentation stage. The advantage of immobilizing yeast is to retain highly concentrated yeast during the continuous fermentation phase, resulting in faster process times, and potentially operating the fermentations at throughputs higher than the nominal washout rate (Masschelin et al.,

Correspondence concerning this article should be addressed to R. J. Neufeld at neufeld@chee.queensu.ca.

1994). The goal then is to reduce processing time without sacrificing product quality.

Common immobilization methods include physical entrapment in a gel matrix or within a membrane-bound microcapsule, adsorption or covalent attachment to preformed carriers, and self-aggregation or crosslinking of cells. A variety of gel matrices have been used for the physical entrapment of whole cells including alginate, agarose, and κ-carrageenan.

Various methods have been reported for the production of cell-loaded gel beads. For industrial large-scale production, the method with the highest capacity is probably the rotating atomizer because of its high throughput. This method was initially designed for alginate gel beads, where gel-inducing calcium would diffuse into the polymer droplets, once caught in a gelation bath. Alginate beads may be unstable in some fermentation media because of the presence of calcium chelators. For brewing purposes, it is then interesting to replace alginate by κ-carrageenan, given that potassium replaces calcium as the gelling cation. Preliminary trials at the pilot scale (200 L of polymer) indicated that the high viscosity of this polymer was not easily compatible with atomizing nozzles.

κ-Carrageenan, like alginate, is a food-grade material, and has been favored for whole cell immobilization because of its superior mechanical strength over that of other gels. Carrageenan consists of alternating 1,3-linked β-D-galactose and 1,4-linked 3,6-anhydro-α-D-galactose. Among the different types of carrageenan, κ-carrageenan is considered the most suitable polymer for cell encapsulation (Rinaudo, 1988). Yeast entrapped in κ-carrageenan gel have been used successfully in the brewing industry for continuous primary fermentation (Linko and Kronlöf, 1991; Mensour et al., 1996). To be a realistic alternative to traditional free cell fermentation and maturation systems, immobilized cells must be stable for relatively long operational times, characteristically measured in weeks or months. Mass transfer limitations of substrate into, and products out of, the immobilized cells and associated matrix are also of critical interest (Nava Saucedo et al., 1994).

The process described in this study involves the formation of an emulsion between a nonaqueous continuous phase (vegetable oil) and an aqueous dispersed phase (κ-carrageenan previously inoculated with yeast), with the use of static mixers. The dispersed emulsified carrageenan-cell droplets undergo rapid gelation by chilling the emulsion. Gel beads are then recovered from the oil by filtration, screening, decanting, or phase partitioning, and the oil may then be recycled back into the process. For brewing operations, resultant beads must be fully clean of the oil, after the phase-partitioning step.

Static mixers consist of a series of stationary elements placed transversely in a tube, as illustrated in Figure 1. The elements form crossed channels that cause the repeated division, rotation, and the longitudinal recombination of the liquid flowing through the static mixer. When two immiscible fluids are pumped through the mixer, the transverse rupture of fluid streamlines into an increasingly homogeneously dispersed emulsion is promoted (Mutsakis and Robert, 1986). Static mixers enable the formation of a fine emulsion with controllable properties in flows similar to those encountered at an industrial scale (several to hundreds L h^{-1}). Furthermore, as it operates in continuous mode, on-line control of bead properties through adjustment of the operational parameters is possible.

A variety of static mixers are commercially available with varying geometry and special applications. Increasingly, static mixers are replacing dynamic systems because rotating equipment consumes power, requires routine maintenance, and can be a significant investment. The static mixer on the other hand, fits in-line, has no moving parts and no electrical requirements, other than the pumping power required to move the fluids (Myers et al., 1997).

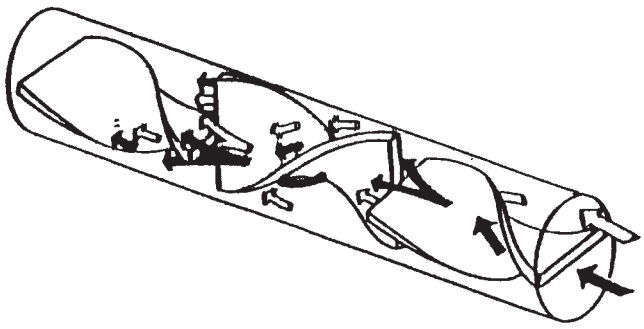

Figure 1. Static mixer similar in design to the Kenics mixer used in study.

Individual mixer elements are shown mounted in tube. Diameter of mixer elements equals that of the inner diameter of tube.

Four operating parameters were selected in this study to determine their influence on the gel bead size distribution: the diameter (D) and the number of elements (N_e) of the static mixer, the linear velocity of the mixture (V), and the volumetric fraction of κ-carrageenan (ϵ). The objective of the work consisted in a demonstration of the use of static mixers operating in a continuous mode, generating emulsions yielding yeast-loaded beads. Second, the operating conditions yielding an appropriate bead mean diameter and size distribution were evaluated. Smaller bead diameters are preferred, reducing intracapsular mass transfer limitations. The potential of this immobilization technology in terms of productivity is also assessed to establish that it meets the demands of industrial-scale operations.

Materials and Methods

Reagents

κ-Carrageenan (type x-909, lot 330360, Copenhagen Pectin, Denmark) is a thermogelling polysaccharide extracted from red algae. Its gelation temperature depends on the concentrations of both κ-carrageenan and potassium chloride (KCl). The polymer was dissolved at 80°C to a concentration of 30 g L^{-1} in distilled water containing 1.5 g L^{-1} of KCl. Under these conditions, the gelation temperature is 28°C. The solution was mixed in a 40-L bioreactor (New Brunswick Scientific Co., Edison, NJ), maintained at 80°C for 20 min to completely dissolve the polymer, then sterilized at 121°C for 30 min, and finally cooled and maintained at 40°C.

A commercial grade of canola oil (Pasquale Bros., Weston, Ontario, Canada) was sterilized for 1 h at 121°C and stored at room temperature (20°C).

Kenics static mixers

Kenics static mixers (Figure 1) were purchased from Cole-Parmer Instrument Company (Niles, IL). Three mixer diame-

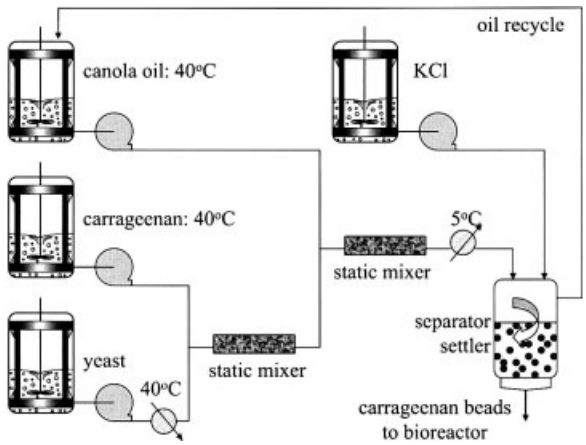

Figure 2. Process developed to formulate yeast-loaded carrageenan microspheres using continuous flow, static mixer technology.

ters (6.4, 9.5, and 12 mm) were tested. The elements were fixed inside tubes with an internal diameter equivalent to that of the static mixer diameter. The number of elements was varied from 12 to 120.

Bead production process

The 40°C sterilized polymer was pumped (Masterflex peristaltic pump, model 07J23-20; Cole-Palmer, Chicago, IL) to a first static mixer, as shown in Figure 2, and mixed with 40°C inoculum (10^7 cells per mL water), before passing through the mixer. The dispersion of the inoculum into the carrageenan solution was achieved by means of the first mixer. The time of contact between yeast and warm polymer solution was minimized so as to avoid loss of viability. This was confirmed experimentally.

The inoculated carrageenan solution was then mixed with the canola oil (40°), after which it passed through the second static mixer to form the carrageenan/oil emulsion, in which the oil becomes the continuous phase. The resulting emulsion was rapidly cooled to 5°C, initiating gelation of the polymer droplets. The emulsion was mixed with cold sterile 22 g L^{-1} KCl in a separator settler, and the beads were recovered by phase partitioning to the KCl solution where gelation was completed. The process oil was recycled back to the oil feed tank and the aqueous bead suspension transferred to a holding tank before loading into a gaslift bioreactor.

Sterilization of the various lines, including the static mixers, and tanks was achieved with 121°C steam for 1 h. Accumulated condensate was cleared with sterile compressed-air injections.

Bead diameter measurement

Beads were sampled into 22 g L^{-1} KCl and stored at 4°C before analysis. Bead diameter was measured by placing beads in a petri dish containing a thin film of water, observing them with a video camera (Pentax macro 50 mm with possible enlargement to 30 and 80 times), and analyzing the image with Optimas image analysis software (Version 4.02, Bioscan, Inc., Washington, DC). Some 300 to 400 beads were measured per sample. The Optimas software package capability lies between 100 μm and several mm, with a maximum absolute error of 30 μm.

The size distributions were analyzed on a number-frequency (%/μm) vs. bead diameter curve and characterized by its mean diameter (d_m), standard deviation (σ), and coefficient of variability (CV = σ/d_m).

Experimental design

In the experimental design, a total of 98 experimental trials were run, by varying process conditions, and evaluating the resulting bead mean diameter (d_m), standard deviation (σ), and coefficient of variability (CV). Of the 98 trials, 38 were run in triplicate, giving a total of 174 trials. Experiments were grouped into two subsets of trials. In the first subset, the following conditions were tested:

- Series of 6, 12, 24, 48, 60, 72, and 120 elements (N_e) within the static mixer were tested.
- Linear fluid velocity (V) through the mixer was varied from 1.7 to 23.8 cm s^{-1}.
- Static mixer diameter (D) was constant at 12.7 mm.
- Volumetric fraction of the κ-carrageenan solution to oil (ϵ) was constant at 12.5%.

In the second subset:

- Volumetric fraction of the κ-carrageenan solution (ϵ) was varied from 8.3 to 50%.
- Linear fluid velocity (V) was varied from 1.7 to 23.8 cm s^{-1}.
- Static mixer diameters (D) of 6.4, 9.5, and 12.7 mm were tested.
- Number of elements (N_e) was held constant at 24.

Liquid linear velocity (V) in the static mixer was calculated as the sum of the oil and κ-carrageenan volumetric flow rate divided by the mixer cross-sectional area.

Results

Beads produced by the static mixer were characterized by a unimodal size distribution. Satellite peaks were not apparent, and distribution data were fitted to a normal law curve calculated with the sample mean and standard deviation (Figure 3). The Kolmorogof–Smirnov method (Sheaffer and McClave, 1990) was used to confirm normality. Mean diameters of the

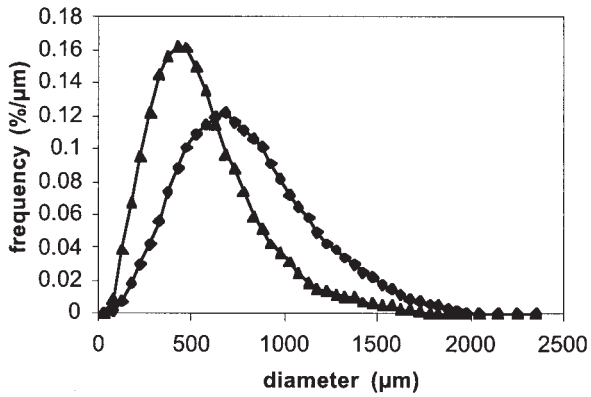

Figure 3. Typical bead size distributions determined under the following process conditions: (▲) V = 13.2 cm s^{-1}, D = 9.5 mm, N_e = 24, ϵ = 25%; (◆) V = 3.5 cm s^{-1}, D = 9.5 mm, N_e = 24, ϵ = 25%.

two typical bead preparations illustrated in Figure 3 were 558 ± 142 and 811 ± 190 μm, respectively. Beads were spherical, discrete, and ranged in diameter from 20 μm to about 2 mm.

The mean size d_m and standard deviation σ values, obtained from triplicate samples of the same treatment, were calculated for 38 of the 98 experimental trials. For the whole experimental design, the error of d_m values ranged between 10 and 70 μm, whereas the error of standard deviation σ values ranged between 10 and 40 μm. These variations were similar to the absolute error generated by the image analysis system (30 μm). Therefore, it can be concluded that the process generated reproducible size distributions.

The number of stationary mixing elements in the static mixer N_e effectively defines the mixing time of the emulsion. A small number of elements can lead to incomplete emulsification, whereas too large a number would constitute a loss of energy and investment. Between 6 and 120 elements were tested. Six elements were not sufficient to achieve good emulsification, and 12 elements were insufficient at low linear velocity ($<$7 cm s^{-1}). For a larger N_e, the bead mean diameter d_m decreases asymptotically, both with N_e and with V, as seen in Figure 4a. It is evident that appropriate choice of N_e and/or V would provide considerable control over the resulting mean diameter of the immobilized cell beads. The range of d_m values possible, under the range of configurations described by Figure 4, runs from 480 to 1211 μm.

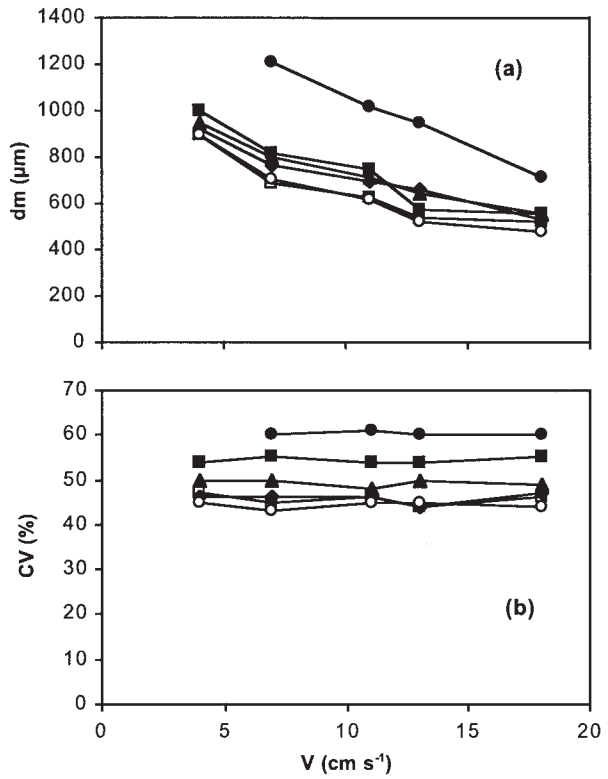

Figure 4. Impact of the linear fluid velocity (V) and the number of mixer elements (N_e) on the mean diameter d_m (a) of the resulting microspheres and on the coefficient of variability CV (b).

D = 12.7 mm, ϵ = 12.5%, and N_e **values of 12 (●), 24 (■), 48 (▲), 60 (◆), 72 (□), and 120 (○).**

Increasing the linear liquid velocity V, through the static mixer, provides more mixing energy; thus the emulsion droplet diameters are inversely proportional to the energy required to break droplets. Thus as the droplets become smaller (decrease in d_m), the energy required to further break up increasingly smaller droplets increases to some higher power of the droplet diameter. Generally, the effect is more noticeable at low V, than at high V, as the bead diameter d_m asymptotically approaches a minimum, as seen in Figure 4a. The linear liquid velocity V would also affect the mixing time and mixing intensity, in a manner similar to that of increasing N_e.

The coefficient of variability CV also decreases asymptotically with N_e, but is independent of V, as shown in Figure 4b. The CV ranged from a low of 43% with N_e of 120, to a high of 61%, with the smallest number of mixing elements (N_e = 12).

While increasing the number of elements (effectively, the mixing time), the emulsification equilibrium is approached as a first-order kinetics, in good agreement with observations both for d_m and the CV. In subsequent experiments, it was assumed that 24 elements would constitute a good compromise between efficiency and cost.

In the second set of experiments, the impact of κ-carrageenan volumetric fraction ϵ and static mixer diameter D were studied over a range of linear fluid velocity through the mixer. For volumetric fraction (carrageenan–yeast/oil) at or above 50%, the dispersed and continuous phases were inverted, resulting in the inclusion of oil droplets within a carrageenan sol. At the other end of the scale, the productivity was considered unacceptably low at $\epsilon < 8\%$.

Figure 5a illustrates a linear decrease in d_m with increasing V through the mixer at D = 9.5 mm. Bead mean diameters were similar at all carrageenan ϵ values, except that beads formed at higher ϵ values deviated increasingly from linearity at low linear flow velocities V. The largest deviation from linearity occurred at ϵ = 50%, at which the carrageenan volume fraction approached the inversion point of the emulsion. In all cases, the number of mixer elements N_e was fixed at 24. The results were similar (data not shown) for mixer diameters of 6.4 and 12.7 mm, in which the bead mean diameters d_m were similar for all values of carrageenan volumetric fraction ϵ, with upward deviations from linearity at low volumetric flow rate V, particularly at high ϵ.

The coefficient of variability for the data shown in Figure 5a is illustrated in Figure 5b. CV values covered a fairly narrow range (45–57%), without a particular trend evident, either with ϵ or with V.

The effect of the static mixer diameter D on the resulting bead mean diameter d_m is illustrated in Figure 6, for three mixer diameters and carrageenan volumetric fractions ranging from 8.3 to 50%. It is clear that the effect of increasing D is to increase d_m, at the same liquid linear flow rate V. The effect is observed over most of the range of V tested, although the differences become smaller at higher V. The trend observed in Figure 5, with d_m decreasing linearly with V, particularly at higher V, is also observed in Figure 6. Upwardly increasing deviations from linearity are seen at lower V. The range of d_m values possible with variations in D and V are considerable, extending from less than 400 μm, to beads with mean diameters approaching 1 mm.

The coefficient of variability for the data shown in Figure 6 is illustrated in Figure 7 for a representative set of data (N_e =

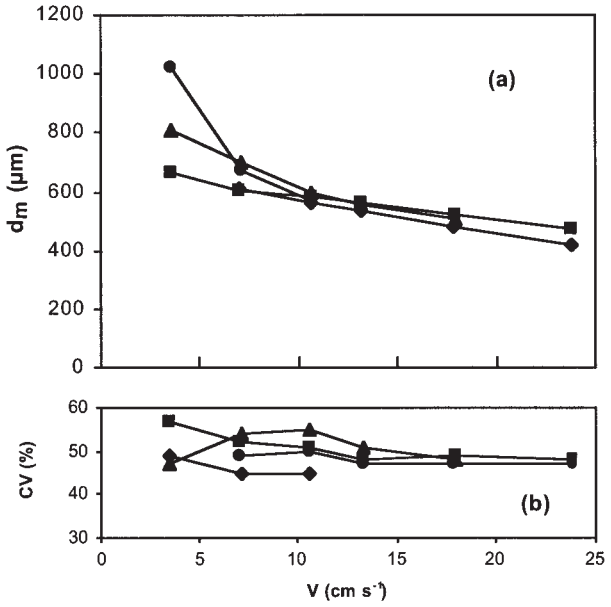

Figure 5. Effect of the linear fluid velocity (*V*) and the volume fraction of carrageenan ϵ, on the resulting microsphere mean diameter d_m (a), and on the coefficient of variability CV (b).

D = 9.5 mm, N_e = 24, and ϵ values (in %) of 8.3 (●), 12.5 (■), 25 (▲), and 50 (◆).

24; ϵ = 8.3%). Results for the other cases (ϵ values of 12.5, 25, and 50%) were similar to those illustrated in Figure 6. The CV for ϵ of 8.3% remained relatively stable with increasing V, and it is evident that the smaller mixer diameters resulted in the lowest levels of bead size distribution, as measured through the CV. For example, the CV values for a 6.4-mm mixer diameter ranged from 36 to 44%, whereas CV values for a 12.7-mm mixer diameter ranged from 54 to 65%.

Discussion

There are few immobilization technologies available for the encapsulation of living cells, on a scale required for industrial application. This is particularly true when it is desired to formulate smaller diameter beads, with mean diameters in the tens or hundreds of microns. Single droplet formulation techniques are common in the laboratory, but are generally not appropriate for industrial use because of the high viscosity of the gelation polymers, making the production of large batches of small-diameter beads impractical. Emulsion technologies show promise because they are well suited to large-scale, continuous production and are best suited for the formulation of smaller-diameter beads. The drawback with emulsion–gelation or emulsion–polymerization technologies is that a solvent or oil phase is required for dispersion, requiring a more difficult phase-separation step than that required when using droplet extrusion methodologies. Second, emulsions involve breakup and coalescence of droplets, leading to characteristic droplet size distributions. Sorting beads by diameter through sieving leads to wastage of expensive encapsulant; thus if a size distribution can be tolerated, large batches of controlled mean diameter beads may be formulated when required for processes such as that required for continuous brewing operations.

The characteristic size distribution obtained with a Kenics-type static mixer used in the present study is unimodal, without apparent satellite peaks. Poncelet et al. (1992) observed primary, secondary, and satellite peaks corresponding to beads

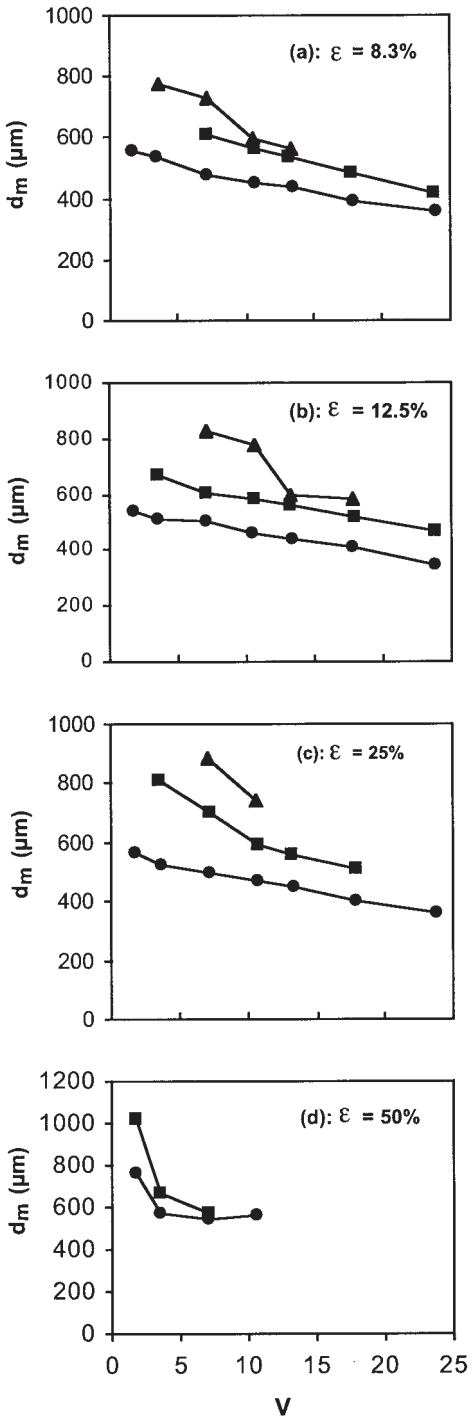

Figure 6. Impact of the linear fluid velocity *V* and static mixer diameter *D* on the microbead mean diameter d_m. N_e = 24.

Results are shown for carrageenan volumetric fraction ϵ values (in %) of 8.3 (a), 12.5 (b), 25 (c), and 50 (d). Static mixer diameters used were D values (in mm) of 6.4 (●), 9.5 (■), and 12.7 (▲).

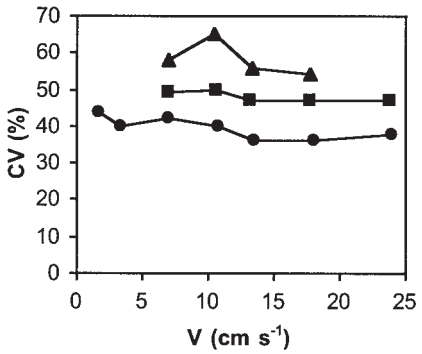

Figure 7. Effect of linear fluid velocity *V*, with varying static mixer diameter *D*, on the microbead coefficient of variability CV.

$N_e = 24$, $\epsilon = 8.3\%$, and D values (in mm) of 6.4 (●), 9.5 (■), and 12.7 (▲).

with diameters < 200 μm, in alginate microspheres produced by emulsion dispersion in a batch stirred tank. With the static mixer, it is possible that very small microspheres, corresponding to the satellite peak region in the case of alginate formed in a stirred tank, are simply washed out during phase transfer and washing, and thus may not appear in the size distribution. In this study, beads were formed with mean diameters ranging from a low of 350 to a high of 1200 microns. Because of the diameter range in the size dispersion, beads were produced with diameters ranging from 20 microns to 2 mm. Thus emulsion technologies provide considerable flexibility in choice of mean diameter, as long as the size distribution can be tolerated.

Several parameters were shown to have an influence on bead mean diameter, including linear fluid velocity V, static mixer diameter D, and carrageenan sol volume fraction ϵ. Within this combination of emulsification parameters, considerable flexibility in the choice and control of mean bead diameter is possible.

The energy required to form an emulsion is proportional to the interfacial area created between the polymer aqueous dispersed phase and the continuous oil phase. Thus, the smaller the bead size, the larger the energy required for their formation. Berkman and Calabrese (1998) showed that an increase in the average fluid velocity through a static mixer would promote an increase in the dissipated energy per unit fluid mass, thus favoring a reduction in emulsified droplet size. This was evident in the present study because an increase in the linear velocity resulted in a decrease in the bead mean diameter. Increases in fluid flow result from a pressure differential across the mixer, proportional to the dissipated energy per unit mass of liquid. An increase in velocity therefore induces an increase in dissipated energy, favoring a reduction in bead size. However, the mean bead diameter reduces toward a minimum at equilibrium, at higher fluid velocities. Taweel and Walker (1983) showed that a dynamic equilibrium is established between the formation of droplets (beads) and the coalescence between droplets for high velocities corresponding to significant turbulence levels. For constant mixer diameter D, carrageenan volume fraction ϵ, and number of mixer elements N_e, the fluid superficial velocity had no effect on the coefficient of variability. Fluid velocity is thus a factor that uniquely affects the average bead diameter, but has little or no effect on the size dispersion.

At low fluid velocities, an increase in κ-carrageenan volumetric fraction ϵ would favor the coalescence between emulsified droplets, thus entailing an increase in bead mean diameter and size dispersion. Over most of the range of fluid velocity V tested, carrageenan volumetric fraction ϵ did not affect the bead mean diameter d_m or the size dispersion CV. An increase in the volumetric fraction of polymer solution, at high fluid velocities, would therefore not increase the rate of coalescence between emulsified droplets. For the 12.7-mm-diameter static mixer, a maximum ϵ of 25% was studied because of pump limitations. If these results were confirmed for larger mixers, increases in productivity would be conceivable. Audet and Lacroix (1989) studied this parameter extensively for the production of κ-carrageenan beads in vegetable oil using a biphase dispersion in a stirred tank and concluded that ϵ had no effect on the mean bead diameter for a κ-carrageenan concentration of 3%. The results with a stirred tank and a static mixer were thus similar in this respect.

An increase in the static mixer diameter D would result in a greater range of shear forces at the same linear fluid velocity, increasing the size dispersion of the emulsified droplets, and thus the resultant beads. An increase in D would also decrease the overall intensity of the shear forces, thus increasing the mean bead diameter. Both of these effects were observed in the present study. The pressure differential across the static mixer was not measured in this study, but it would seem to play a role in controlling V, and thus understanding the effect of D in combination with V, on both bead diameter and size distribution.

Increasing the number of mixing elements in the static mixer results in increased emulsification time because of the extended residence time within the mixer. An increase in the number of mixing elements also increases local fluid velocities, and thus the intensity of mixing, while minimizing the large shear gradients typical of mechanically mixed vessels such as those using turbine impellers. A more homogeneous mixture was expected as a consequence of a reduction in the size of the larger beads, ultimately leading to a reduction in d_m and CV. Experimentally, an equilibrium was achieved at between 60 and 72 mixer elements, where no further reduction in d_m or CV was observed. Middlemen (1974) showed that 10 elements were sufficient to attain such an equilibrium in the case of emulsions made up of low-viscosity constituents (0.6 to 1 cp). The κ-carrageenan solution used in these experiments (30 g L^{-1}) had an average viscosity of 200 cp, whereas the oil viscosity was 25 cp. The large difference in the number of mixing elements needed to reach an equilibrium can be explained by the high-viscosity polymer that would inevitably require a longer mixing or residence time to reach equilibrium.

The objective for the purpose of manufacturing yeast-immobilized gel beads for continuous brewing operations is to operate the static mixer at high productivity while formulating gel beads with a size distribution between 200 μm and 1.6 mm. In a related study, inoculated bead batches were produced (about 25 L) to feed a continuous bioreactor. The results obtained demonstrated that the primary beer fermentation time could be reduced from the classical batch fermentation of 6 to 7 days, to 20 h (unpublished data). Assuming a static mixer diameter of 12.7 mm, a κ-carrageenan volume fraction of 25% and a production of 740-μm-diameter beads, one would obtain a productivity of nearly 10 L h^{-1} beads. Larger production volumes could be obtained by increasing the mixer diameter.

For example, with a 5-cm static mixer, the productivity can exceed 200 kg h^{-1}. Producing smaller beads will require a higher fluid linear velocity and, thus, a higher productivity. Reducing the bead size, from 600 to 300 μm, would double the productivity. The opposite situation is encountered in more conventional bead production approaches using droplet extrusion technology (Poncelet et al., 2000).

An increase in productivity is necessary to operate at an industrial scale. Consequently, an increase in the flow of polymer and oil with the static mixer used in this study would induce the formation of beads too small to be used in the fermentation stage. It is therefore necessary to increase the diameter of the static mixer, thus increasing the diameter of the beads. However, if the results obtained in this study can be extrapolated, the use of static mixers with larger diameters will increase the bead size dispersion, producing a larger percentage of beads outside the set guidelines. Another possible alternative would be the implementation of a system using multiple static mixers of medium size (12.7 mm) placed in parallel. Productivity reaching 100 kg h^{-1} (with 10 mixers, 600 μm beads) is therefore conceivable. Another solution would be to study the characteristics of different static mixer designs to determine their efficiency.

In summary, both the static mixer diameter and the carrageenan volumetric fraction affect the bead mean size but mainly in correlation with the fluid linear velocity through the mixer. This last parameter remains the most important factor, followed by the static mixer diameter. With respect to the size dispersion, the coefficient of variation is mainly and only affected by the static mixer diameter. This observation raises concerns about scale-up. Although the coefficient of variation is relatively good for smaller-diameter static mixers (35%, similar to that obtained with turbine mixers), it increases with the mixer diameter, and thus is negatively affected on scale-up.

It has been shown that yeast-loaded carrageenan beads, with controlled diameter and tight size distribution, can be produced on a continuous basis, in a scalable mixer, at production rates appropriate to pilot plant–scale and, potentially, industrial-scale operations. The continuous production of alcohol using the immobilized cells in a fluidized bed reactor will be the subject of subsequent reports.

Acknowledgments

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Notation

D = static mixer diameter, mm
N_e = number of elements
V = superficial fluid velocity, cm s^{-1}
ϵ = volumetric fraction of κ-carrageenan, %
d_m = bead mean diameter, μm
σ = standard deviation, μm
CV = coefficient of variability, %

Literature Cited

Al Taweel, A. M., and L. D. Walker, "Liquid Dispersion in Static In-Line Mixers," *Can. J. Chem. Eng.*, **61**, 527 (1983).

Audet, P., and C. Lacroix, "Two Phase Dispersion Process for the Production of Biopolymer Gel Beads: Effects of Various Parameters on Bead Size and Their Distribution," *Proc. Biochem.*, **24**, 217 (1989).

Begin, A., F. Castaigne, and J. Goulet, "Production of Alginate Beads by a Rotating Atomizer," *Biotechnol. Tech.*, **5**, 459 (1991).

Berkman, P. D., and R. V. Calabrese, "Dispersion of Viscous Liquids by Turbulent Flow in a Static Mixer," *AIChE J.*, **34**, 602 (1998).

Castillo, E., M. Rodriguez, L. Casas, R. Quintero, and A. Lopez-Munguia, "Design of Two Immobilized Cell Catalysts by Entrapment on Gelatin: Internal Diffusion Aspects Enzyme," *Microbiol. Biotechnol.*, **13**, 127 (1991).

Galasso, J. L., and J. E. Bailey, "In Vivo Nuclear Magnetic Resonance Analysis of Immobilization Effects on Glucose Metabolism of Yeast *Saccharomyces cerevisiae*," *Biotechnol. Bioeng.*, **26**, 217 (1983).

Linko, M, and J. Kronlöf, "Main Fermentation with Immobilized Yeasts," *Proc. European Brewery Convention Congress*, pp. 353–360 (1991).

Luong, J. H. T., "Cell Immobilization in κ-Carrageenan for Ethanol Production," *Biotechnol. Bioeng.*, **25**, 2910 (1983).

Masschelein, C. A., D. S. Ryder, and J. P. Simon, "Immobilization Cell Technology in Beer Production," *Crit. Rev. Biotechnol.*, **14**(2), 155 (1994).

Matulovic, U., D. Rasch, and F. Wagner, "New Equipment for the Scaled-up Production of Small Spherical Biocatalysts," *Biotechnol Lett.*, **8**, 485 (1986).

Mensour, N. A., A. Margaritis, C. L. Briens, H. Pilkington, and I. Russell, "Application of Immobilized Yeast Cells in the Brewing Industry," *Immobilized Cells: Basics and Applications*, R. H. Wijffels, R. M. Buitelaar, D. Bucke, and J. Tramper, eds., Elsevier Science, Amsterdam, pp. 661–671 (1996).

Middelman, S., "Drop Size Distributions Produced by Turbulent Pipe Flow of Immiscible Fluids through a Static Mixer," *Ind. Eng. Chem. Process Des. Dev.*, **13**, 78 (1974).

Mutsakis, M., and R. Robert, "Advances in Static Mixing Technology," *Chem. Eng. Prog.*, 42 (June 1986).

Myers, K. J., A. Bakker, and D. Ryan, "Avoid Agitation by Selecting Static Mixers," *Chem. Eng. Prog.*, 28 (June 1997).

Nava Saucedo, J. E., B. Audras, S. Jan, C. E. Bazinet, and J.-N. Barbotin, "Factors Affecting Densities, Distribution and Growth Patterns of Cells inside Immobilization Supports," *FEMS Microbiol. Rev.*, **14**, 93 (1994).

Ogbonna, J. C., M. Mastumura, T. Yamagoata, H. Sakuma, and H. Kataoka, "Production of Microgel Beads by a Rotating Disc Atomizer," *J. Ferment. Bioeng.*, **68**, 40 (1989).

Poncelet, D., S. Desobry, U. Jahnz, and K. Vorlop, "Immobilization at Large Scale by Dispersion," *Springer Lab Manual*, *Immobilized Cells*, R. H. Wijffels, ed., Springer-Verlag, Berlin, 139 (2001).

Poncelet, D., R. Lencki, C. Beaulieu, J. P. Halle, R. J. Neufeld, and A. Fournier, "Production of Alginate Beads by Emulsification/Internal Gelation," *Appl. Microbiol. Biotechnol.*, **38**, (1992).

Poncelet, D., B. Poncelet de Smet, C. Beaulieu, and R. J. Neufeld, "Scale-up of Gel Bead and Microcapsule Production in Cell Immobilization," *Fundamentals of Animal Cell Encapsulation and Immobilization*, M. F. A. Goosen, ed., CRC Press, Boca Raton, FL (1993).

Rehg, T., C. Dorger, and P. C. Chau, "Application of an Atomizer in Producing Small Alginate Beads for Cell Immobilization," *Biotechnol Lett.*, **8**, 111 (1986).

Rinaudo, M., "Gelation of Ionic Polysaccharides," *Gums and Stabilizers for the Food Industry 4*, G. O. Philips, P. A. Williams, and D. J. Wedlock, eds., IRL Press, Oxford, UK, pp. 119–134 (1988).

Scheaffer, R. L., and P. C. Chau, "Probability and Statistics for Engineers," PWS-Kent Publishing, Boston, MA, p. 348 (1990).

Scott, C. D., "Techniques for Producing Monodispersed Biocatalyst Beads for Use in Columnar Bioreactors," *Ann. N. Y. Acad. Sci.*, **501**, 487 (1987).

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