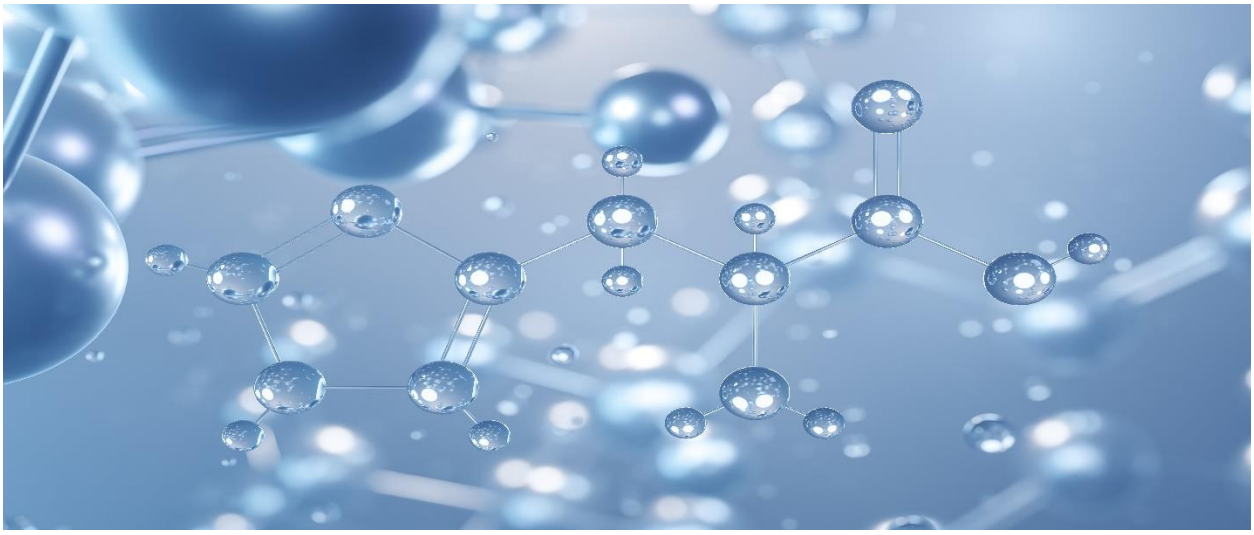
Enzyme immobilization using static mixers

Rakhi Vishwakarma, Marcel Suhner, Kishor Kulkarni



Enzymes are used primarily in the manufacturing of various food products, e.g., wine, yogurt, beer, cheese, vinegar, and sourdough, as well as in the manufacturing of products such as textiles, linen, leather, etc. The need of sustainable processes has augmented the enzymes as catalysts application in industrial divisions. From past decades, the use of enzymes in industries which include baking, starch transformation, beverage production has expanded considerably. Enzymes also play a major role in the textile industry for purification and general manufacturing processes. The application of enzymes is now integral to cosmetics, healthcare, paper making, detergent industries, chemical manufacturing, biofuel manufacturing, and in the recycling and treatment of wastewater.[1]

The high demand of enzymes in various domains hampered the shelf life, stability, and sensitivity of enzymes to many processes' parameters. Hence, immobilizing techniques could help preventing these disadvantages. Enzyme immobilization techniques are encapsulation, adsorption, covalent binding, etc. Depending on the mode of immobilization, the carrier plays a significant role in success of the process. [1]

Static mixer over turbine reactor

The most widely used immobilization material is K-Carrageenan, extracted from marine red algae. Encapsulation protocols for K-Carrageenan hydrogels required for immobilization are based on the emulsification of sol into a hydrophobic phase. Droplets of the emulsified sol are converted to gel by decreasing the temperature. Generally, baffled turbine reactors are used for the batch-wise emulsification, but also the static mixing technology is alternatively used already at a large scale. An important benefit of this technology is the possibility for

continuous processing, which saves time and energy. The standard procedure for the K-Carrageenan production in the form of beads using static mixers involves heating the continuous oil phase and K-Carrageenan sol before mixing and cold oil injection at the outlet of the static mixer to start gelation. A study concludes the K-Carrageenan beads formation by emulsifying gelification at ambient temperature in a continuous process employing Sulzer static mixer technology an increased encapsulation efficiency by two times (Figure 1). Sauter diameter was 300 µm. [2]

Another example of enzyme immobilization using static mixer is to be found in the dairy industry. There is a demand for lactose reduced dairy products as nearly 70% of the world's population is lactose intolerant. Hence, each year 3.2 million tons of lactose dissolved in whey is accumulated and not further processed industrially. The lactose is disposed of as waste and needs to be broken down as it poses an environmental hazard due to its low biodegradability. This is a major problem faced by dairy industry and requires improving the hydrolysis process. The lactose hydrolysis is carried out by an enzyme catalyzed reaction at mild temperature and pH. β-Galactosidase is commonly used for enzymatic conversion. However, it degrades during storage and hence an encapsulation of enzyme is necessary to protect the activity of β-Galactosidase. Emulsion is formed by mixing enzyme/encapsulation solution in a non-aqueous phase such as vegetable oil. Usually, turbine stirred tanks are used for the emulsification, but an alternative approach is to replace them with static mixers (Figure 1) - beneficial for continuous processing at lower Capex and Opex costs.[3]

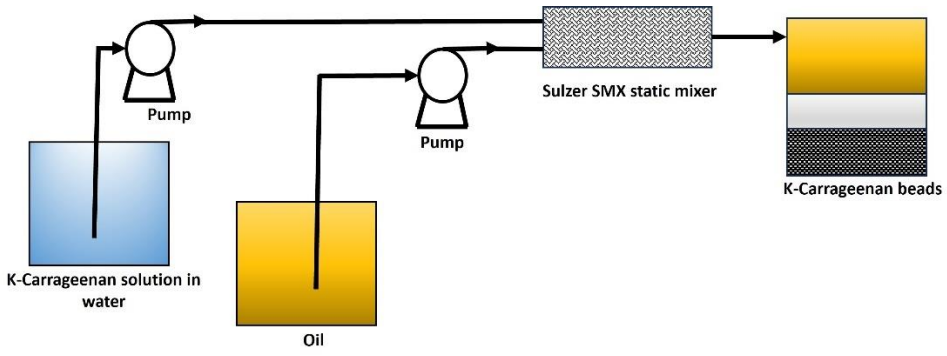


Figure 1. K-Carrageenan beads production using static mixer[2,3]

Short residence time

Emulsification- or residence time could be as short as 2 seconds with low shear stress. The high dispersion power of Sulzer static mixer – depending on the flow rate - gives fine emulsion within seconds in comparison with turbine mixers which takes around 5 – 15 minutes.[2] Sulzer SMX static mixer was used for formulation of hydrogel beads by emulsification. Dispersion with in-situ gelation of K-Carrageenan droplets was attained with the short residence time in the static mixer (1-2 s).[4]

Scale-up

The scale-up with static mixers is straightforward. By increasing the tube diameter, even for viscous components, and keeping certain process parameters constant, similar results as for the test size can be achieved. Also, the cleaning of static mixer is just to flush/backflush with water/ solvent as required by the process, whereas the cleaning and filling of batch reactors decrease the productivity. The limitation for batch reactor scale-up is tedious when expecting reactor sizes bigger than 1cubic meter.[2]

Stability

Beads with cross-linked α-chymotrypsin prepared by static mixers had no activity loss when stored at least for 2 weeks.[2] The results obtained using a Sulzer static mixer show that the stability of the encapsulated enzyme is maintained and can be reused without significant loss of activity. Moreover, storage times for β-Galactosidase beads were up to 4 weeks after production of the beads.[3]

Sauter diameter

The advantage of the static mixer technology is that it produces smaller beads. The main parameters tested were total flow rate and volumetric ratio of K-Carrageenan to oil. The average bead size formed was in the range of 19 -52 µm at an increase in total flow.[3] The bead size (d_{32}) is shown in the Figure 3 as a function of injection temperature (T_{inj}) and flowrate (Q_t). Bead diameter reduced with an increase in the T_{inj} and Q_t . The resulting beads had a Sauter diameter ranging from 350 to 200 µm and a decrease was observed with the increase in the no. of elements under similar parameters of T_{inj} and Q_t. The increase in T_{inj} and Q_t are not the best methods for decrease in bead size as these parameters can lead to coalescence of oil in the beads(Figure 3). Increasing the mixing element to diminish the beads size is a preferable method. The effect of mixing element design and operating parameters for the microencapsulation using static mixer creates a droplet size distribution with is generally very narrow. [4]

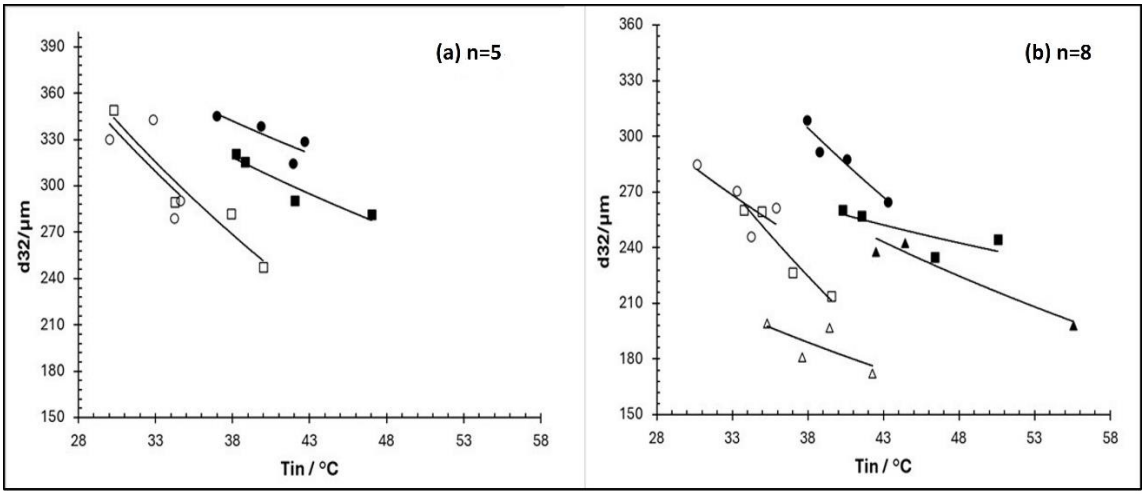


Figure 2. Evolution of Sauter mean diameter d_{32} (a) 5 mixing element (b) 8 mixing element, Tinj: carr/water (open symbols), carr/salt (closed symbols), Qt = 0.2 (circles), 0.3 (squares), 0.4 (triangles) dm3/min, for n = 5 (upper) and 8 (lower) elements in the static mixer[4]

Power requirement and shear

Moreover, only external pumping power is required to operate the static mixer in comparison to the motor of the turbine mixer which drives the impeller. Hence, this factor makes the static mixer a low maintenance appliance saving operational cost. Reaching good emulsification with turbine mixers is linked to high shear that may result in damaging of enzymes during encapsulation such as lipase. [2] Static mixers are gentle on the enzymes and immobilization solutions as they have low shear compared to turbine mixer. Large shear gradients in turbine mixers lead to strong variations of droplet sizes in emulsions. Static mixers can generate small droplet sizes due to the uniform shearing force over the cross section,narrowing down the droplet size distribution. Lower shearing also reduces damage to fragile encapsulants like large molecular biologicals and biochemical materials.[4]

Safety

There is a low risk of mechanical failure and maintenance because there are no moving parts in static mixers. There is a reduced risks for hotspots which can cause exothermic- or runaway reactions as these mixers help to maintain uniform reaction conditions and mixing. Static mixers are promising for enzyme immobilization as the risk of contamination between the batches is minimal which is very important in food processing and pharmaceuticals.

Static mixers find its places in numerous processes including encapsulation of biochemicals and biological materials by emulsification.[2]

This leads to the conclusion that static mixers are economical and enables a continuous process that can be easily scaled-up for enzyme immobilization and found more efficient at mild process parameters.

References:

1. Enzyme immobilization technologies and industrial applications, Y. Maghraby, R. El-Shabasy, A. Ibrahim and H. Azzazy, ACS Omega, 2023, 8, 5184-5196.
2. Immobilization of α-chymotrypsin in κ-carrageenan beads prepared with the static mixer, E. Belyaeva, D. Valle, D. Poncelet, Enzyme and Microbial Technology, 2004, 34, 108-113.
3. Enzyme encapsulation by static mixer method for hydrolysis of lactose, M. Hossain and L. Zhang, International Journal of Biotechnology for Wellness Industries, 2012, 1, 210-221.
4. New approach to the formulation of hydrogel beads by emulsification/thermal gelation using a static mixer, E.Belyaeva, D. Valle, R. Neufeld, D. Poncelet, 2004, 59, 2913-2920.