4
Process engineering and industrial enzyme applications

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4.1 Introduction

Biocatalysis plays an increasingly important role in industrial wet textile pretreatment and finishing processes. Conventional wet textile processes are characterised by long residence times, high concentrations of chemicals, alkaline or acidic pH and high temperatures. It is to be expected that wet textile processes will be shifted considerably towards sustainable processes based on biocatalysis, owing to increasing governmental and environmental restrictions and the decreasing availability of fresh water. Biocatalysis is a flexible and reliable tool that presents a promising technology for fulfilling expected future requirements.

Since the early 1990s a lot of research has been done on reactions catalysed by enzymes that are relevant to the textile industry. Often these studies focus on the enzymatic incubation itself and the enzyme and substrate characteristics and not on parameters necessary for the design of efficient and competitive full-scale industrial processes.

Process parameters need to be related to cloth properties such as the porosity and the density of the fabric in order to introduce efficient and economic enzymatic treatments. The design of enzymatic textile treatment processes is a difficult task, which is often based on trial and error instead of process engineering. On the one hand this is caused by the complex geometrical structure of textile materials and on the other hand by the specific kinetics of enzymatic reactions and the relatively large sizes of enzyme molecules. The time-determining step in the kinetics of these processes is often the transport of molecules to the surfaces of the textile fibres. Although the thickness of textiles is small, in many cases less than 1 mm, the porous structure of the material hinders a free flow of liquid. This means that diffusion of molecules through the pores to the fibre surfaces is the main transport mechanism. This is a relatively slow process, especially if the diffusing molecules are large like enzymes. Therefore much time is needed before the enzyme molecules are adsorbed at all fibre surfaces. It also takes a consid-

erable time before all the reaction products have been removed from porous textiles. In order to say something about the residence time of the fabric in an impregnation step or in a washing out step, the diffusion time of the enzymes in textiles has to be determined.

This chapter gives an overview of different industrial enzymatic cotton pretreatment and finishing processes and focuses on mass transfer in enzymatic wet textile processes. Different possibilities for process intensification are considered and, as well as mass transfer limitation in immobilised enzyme systems, an application of biocatalysis especially relevant in the treatment of the effluents of textile mills is discussed.

4.2 Large-scale industrial enzyme applications in textiles: an overview

The estimated value of the world enzyme market was about US $1.5 billion in 2000 and it has been forecasted to grow to US $2 billion in 2005. In Tables 4.1 and 4.2 large-scale industrial enzyme applications and their market size are summarised (Rehm *et al.*, 1996).

Detergents, textiles, food, starch, paper and pulp, baking and animal feed are the main industries that use approximately 75% of the industrially produced enzymes. The largest manufacturers of industrial enzymes are Novozymes, Genencor, DSM and Röhm & Haas. Detergents have always been the largest application of industrial enzymes. Inventions made in the field of enzyme applications in detergents quite often found their application later in the textile industry. Detergents were also the first large scale application for microbial enzymes. Röhm in Germany had already produced the first commercial enzyme used in a detergent in 1914. Bacterial proteinases are the most important detergent enzymes. Some products have been produced by genetically modified organisms to be more stable or

Table 4.1 Market size of large-scale industrial enzyme applications

Industry	Market size (10^6 US $)
Detergent	500
Textile	150
Drinks/brewing	150
Dairy	150
Pulp and paper	100
Starch	100
Baking	100
Animal feed	80

Table 4.2 Large-scale industrial enzyme applications

Enzyme	Application	Industry	Market size (%)
Protease	Protein degradation	Detergent	50
α-Amylase	Glucose production	Starch	16
	Desizing	Textile	
Cellulase	Colour brightening	Detergent	14
	Fibril removal	Textile	
	Juice extraction	Drinks	
α-Amylase	Shelf life	Baking	11
Lipase	Fat removal	Detergent	7
Pectinase	Juice clarification	Drinks	4
	Scouring	Textile	

active in the hostile environment of the washing machine or the detergent, high temperatures, alkaline pH and oxidising agents.

In the late 1980s lipid-degrading enzymes were introduced in powder and liquid detergents. Lipases hydrolyse ester bonds of fats, thereby producing glycerol and fatty acids. Amylases are used in detergents to remove starch-based stains that stick on textile fibres and bind other stains. Cellulases were introduced in detergents in the early 1990s. Cellulases are able to degrade cellulose and are therefore able to remove cellulose microfibres that are formed during the use and washing of cotton products. The removal of these microfibres by cellulases results in colour brightening and softening of the textile material. Recent developments are in the field of thermostable enzymes, protein engineering and enzymes obtained by genetically modified microorganisms.

The use of enzymes in the textile industry is one of the most rapidly growing fields in industrial biocatalysis (Thiry, 2001). For a long time starch has been used as a protective lubricant and glue of fibres in the weaving of fabrics; amylases are used to remove the starch in the desizing process. Cellulases are used for the enzymatic depilling of cotton fabrics and in the production of denim fabrics. The fading effect on indigo-dyed cotton used to be created by pumice stones, but the pumice stones caused damage to both fibres and machines. The same fading effect is nowadays obtained with cellulase enzymes. An application introduced more recently in the textile industry is the use of enzymes in the cotton scouring process. During scouring, waxes and other hydrophobic material are removed from the cotton fibres. Conventionally this process is done in hot sodium hydroxide (NaOH). Alkaline pectinases are able to degrade pectin in the outer layers of the fibre, thereby weakening the structure of the outer layers so they can be removed afterwards.

4.3 Industrial applications of enzymes in wet textile processing

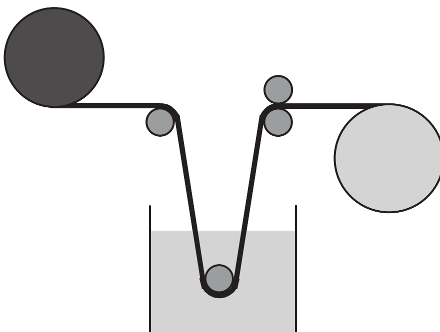
Enzymes are gaining an increasingly important role as a tool in various wet textile pretreatment and finishing processes (Stanescu, 2002; Thiry, 2001; Cavaco-Paulo, *et al*. 1998; Heine and Höcker, 1995). Conventional wet textile pretreatment and finishing procedures applied in the textile industry are often characterised by high concentrations of chemicals, alkaline or acidic pH, and high temperatures with consequent high consumption of energy. Enzymes are very specific catalysts; they operate best at ambient pressures, mild temperatures and often at a neutral pH. It is to be expected that, within 5 to 10 years, wet textile production processing will be shifted substantially towards sustainable processes, because of increasing governmental and environmental restrictions and the decreasing availability of fresh water. Biocatalysis has proven to be a flexible and reliable tool in wet textile processing and a promising technology for fulfilling expected future requirements.

In the scientific literature a lot of detailed information can be found on the different reactions catalysed by enzymes that are relevant to the textile industry, such as desizing, biopolishing, biostoning and more recently bioscouring (for more examples see preceding chapters). Most studies described in scientific literature focus on aspects that are directly related to enzymatic incubation, the enzyme and substrate characteristics (e.g. Agrawal *et al*., 2002; Buchert *et al*., 2000; Buschle-Diller *et al*., 1998; Cavaco-Paulo *et al*., 1996, 1997, 1998b; Etters, 1999; Hartzell and Hsieh, 1998; Lenting and Warmoeskerken, 2001a, 2001b; Lenting *et al*. 2002; Li and Hardin, 1998; Pere *et al*., 2001; Tzanov *et al*., 2001; Yachmenev *et al*., 2001). However, apart from reaction mechanisms, the relationship between substrate and enzyme, the amount of shear or agitation, optimal temperature and pH etc., these studies often do not focus on parameters necessary for the design of true full-scale industrial processes. This is partially caused by the fact that mass transfer and shear, for example, are quite different in laboratory-scale equipment than in industrial batch and (semi-)continuous equipment. Process parameters need to be related to cloth properties such as the porosity and the density of the fabric in order to introduce efficient and economic enzymatic treatments. Most information relevant for the design and development of industrial processes comes from companies producing enzymes or companies that develop formulations and applications for the textile industry (e.g. Bayer, Genencor, Novozymes, Dexter Chemical Corp.) and some from scientific or more technical publications that are dedicated to industrial enzymatic wet textile pretreatment or finishing processes (Lange and Henderson, 2000; Lange, 2000; Cortez *et al*., 2001, 2002; Contreras, 2001; Waddell, 2002).

4.3.1 Desizing of cotton

During weaving, warp yarns are exposed to considerable mechanical strains. To prevent the yarns from breaking, they are coated with a sizing agent (a protective glue and lubricant). The sizing agent is most often based on starch. Apart from starch, synthetic sizing agents are available, for example polyvinyl alcohol (PVALc), but, for economic reasons, starch is still the most favourable sizing agent. After weaving the fabric, the sizing agent needs to be removed since it hinders textile-finishing processes such as dyeing. This desizing process used to be done chemically using, for example, hydrogen peroxide, (H_2O_2) and sodium hydroxide (NaOH), but since the 1950s enzymatic desizing processes based on α-amylases have been widely introduced and implemented successfully in the textile industry. In the enzymatic desizing process an almost complete removal of starch-containing size is obtained without any fibre damage. Amylases were derived from mulds or pancreas but are nowadays produced by bacteria (especially *Bacillus subtilis*). Biotechnological progress and genetic engineering of microorganisms have allowed thermostable enzymes to be widely available nowadays, and therefore different temperatures, ranging from 20 to 80°C for conventional α-amylases and 40 to 110°C for thermostable α-amylases, are applicable. The optimum pH lies between 4 and 10 depending on the enzyme used. Most α-amylases are suitable for all common batch and (semi-)continuous processes, such as jet, jig, cold and hot pad-batch (see Fig. 4.1), pad-steam and J-box. Typical process conditions for woven cotton fabrics are summarised in Table 4.3 (data from product guides and product information sheets from Novozymes, Bayer and Genencor).

During impregnation the hot water causes the starch to gelatinise and the fabric becomes fully wetted and impregnated with the enzyme solution. The average liquid take-up during impregnation is approximately 1 L/kg



4.1 Pad-batch process, typically used in the enzymatic desizing of cotton fabrics.

Table 4.3 Typical process conditions for some common textile desizing applications

	Jig	Winch	Pad-batch (cold)	Pad-batch (hot)	Pad-steam
Impregnation:					
Enzyme dosage (mL/L)	0.3–1	0.3–1	1–10	1–6	1–10
Temperature (°C)	60–95	70–100	15–40	60–70	20–110
pH	5–7.5	5–7.5	5–7.5	5–7.5	5–7.5
Incubation:					
Incubation time	2–4 (passages)	30 (min)	6–24 (h)	3–8 (h)	15–120 (s)
Temperature (°C)	60–100	90–100	15–40	60–70	90–110

fabric, and depends on the characteristics of the fabric such as the porosity and the additives present in the impregnation liquor. Chelating agents should preferably not be used during the desizing process because calcium ions (at ppm level) stabilise the enzymes. Wetting agents and non-ionic surfactants can be used to enhance enzyme penetration and adsorption, fibre swelling and to promote the removal of waxes, soils and synthetic sizing agents. Non-ionic surfactants are suitable for combination with enzymes, whereas anionic and cationic surfactant may inactivate the enzyme through denaturation. Lubricants are generally recommended to be used in combination with α-amylases during desizing, especially in jets and rotary washers, to reduce the formation of crease marks and streaks. After the enzymatic treatment, fabrics should be washed off above 80°C, often between 90 to 100°C, in alkaline liquor followed by a wash in neutral liquor.

4.3.2 Cotton finishing: enzymatic ageing and depilling

The application of cellulases in wet textile processes has been, like enzymatic desizing, successfully introduced and accepted in the textile industry. Cellulase enzymes are a class of hydrolytic enzymes that are used for different cotton finishing processes: cellulases can be utilised to give indigo-dyed cotton fabrics (denim) an aged appearance (also known as biostoning), and to give cotton fabrics a renewed appearance by colour brightening and softening of the material through the removal of microfibres (depilling, also known as biopolishing). As mentioned in Chapter 3, cellulase is a typical multicomponent enzyme that consists of mainly:

- Endoglucanases (EGs), (EC 3.2.1.4);
- Cellobiohydrolases (CBHs) or exo-cellobiohydrolases, (EC 3.2.1.91);
- β-glucosidases or cellobiases (EC 3.2.1.21).

Cellulases can be derived from a variety of microorganisms, especially fungi, such as *Trichoderma reesei*, *Humicola insolens*, *Aspergilus niger* and *Bacillus subtilus*. These organisms can all be used to produce acid-stable as well as neutral- and alkaline-stable cellulase mixtures. Natural cellulase mixtures are produced by microorganisms to hydrolyse insoluble cellulose very efficiently. In textile finishing processes this is not necessary or even undesirable.

Nowadays it is an accepted concept that the performance characteristics in textile finishing applications of a certain cellulase composition are determined by its specific composition, rather than the optimum pH or temperature of the enzymes present in the mixture, or the microorganism used to produce the enzymes. Besides conventional cellulase mixtures, dedicated cellulase compositions are nowadays available commercially, such as EG-enriched cellulase mixtures, monocomponent cellulases and even modified cellulase enzymes with unique performance features, thanks to modern biotechnological techniques.

4.3.2.1 Enzymatic ageing

The finishing of denim garments by pumice stones (stonewashed garments) to achieve an aged or worn appearance has been radically improved by the application of cellulase enzymes (also called cellulase washing or biostoning). This stonewash effect is due to abrasion of the fabric thereby locally removing the surface-bound indigo dye and revealing the white interior of the yarn. In the traditional stonewash process, abrasion is caused by pumice stones and by garments chafing against the washer drum. The pumice stones damage the washer drum and reduce the fabric strength due to abrasion. The application of cellulases prevents damage to the washing machine, eliminates the need for disposal of used stones and results in an improved quality of wastewater because of the absence of pumice stone dust. Owing to the absence of stones (several kilograms) the garment load may be increased up to 50% resulting in increased productivity. The use of cellulases results in a softer fabric and, because of the reduced abrasion of the fabric, an increased strength compared to the traditional stonewash process. The application of cellulases is, owing to the global market size for stonewashed denim garments, a very successful application of enzymes in the textile industry.

EG-enriched or EG monocomponent formulations are usually preferred because of their superior performance in biostoning (see the preceding chapter). Most commercially available formulations are produced from the fungi *Trichoderma reesei* (optimum pH 5) and *Humicola insolens* (optimum pH 6.5–7.0). EGs from *Trichoderma reesei* are known for their great effectiveness, and therefore give a flatter and lower contrast pattern and a small

Table 4.4 Typical process conditions for an industrial biostoning process

Parameter	
pH	4.5–7.0
Temperature	45–65°C
Liquid ratio	3:1–20:1
Incubation time	15–60 min

amount of hydrolysis of the fabric, but have a tendency to promote indigo backstaining (redeposition of indigo dye on the undyed white weft yarns of the fabric). Neutral EGs produced from *Humicola insolens* are known for their low levels of backstaining and the enzymes have a broader pH range. The latter property allows for a more reproducible process. Backstaining is promoted by adsorption of indigo on the enzyme and the subsequent adsorption of the enzyme on to the fabric. The use of enzymes with a low affinity for indigo and the absence of, for example, a cellulose binding domain (CBD) will thus result in a reduced amount of backstaining (see the preceding chapter). Different commercial formulations are nowadays available with specific key features for different applications and results.

An enzymatic stonewash process requires equipment with sufficient shear forces and mixing, such as a drum washer. Typical parameters for commercial formulations are summarised in Table 4.4 (Product Guide, Genencor). The use of a buffer solution is recommended, especially when applying an acidic formulation. Before enzymatic stonewashing, proper and complete desizing is recommended. The incubation time depends on the type of machine, the liquid ratio, the garments or fabrics and the desired effect or look. It is important not to overload the machine, because that will reduce the amount of shear force and mixing.

The enzyme dosage depends on the type, density, porosity and hydrophilicity of the fabric or garment and the effect desired. It is a function of treatment time, pH, temperature, liquid ratio, auxiliary chemicals and the type of equipment (shear force and mixing). In general, the addition of non-ionic surfactants and dispersing agents is recommended and will enhance the overall performance. The cellulase enzymes need to be inactivated after the desired stonewash effect is obtained. Insufficient inactivation will result in extended degradation of cellulose and therefore an undesirable strength loss and weight reduction. There are several options to inactivate the cellulases:

- Increase the pH (pH > 9) and raise the temperature ($T > 60$°C) for 15 minutes;

- Wash off the fabrics in an alkaline detergent solution (pH > 9 and $T > 60°C$) for 15 minutes;
- Perform a standard chlorine bleach of the fabrics.

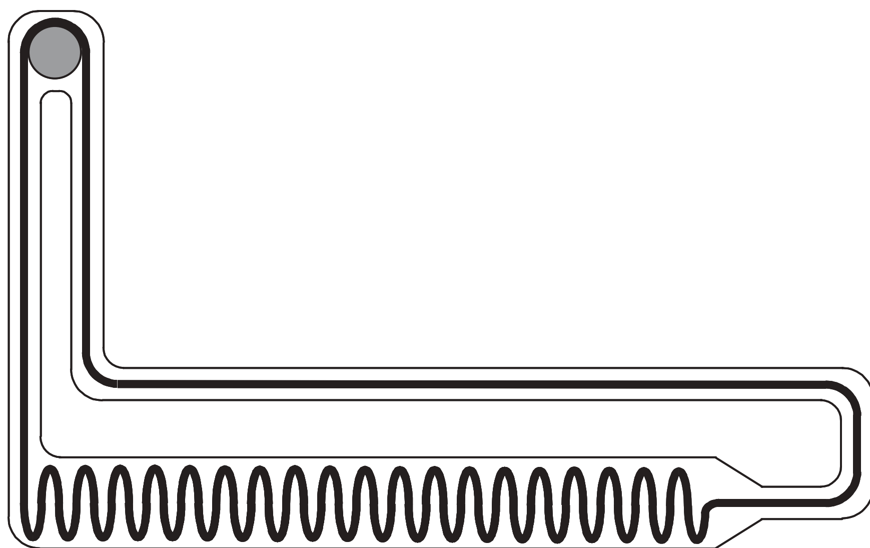
4.3.2.2 Depilling

In cotton fabric, fuzz (microfibres) emerges from the surface. When these microfibres become entangled during processing, pills are formed. Depilling (also called biopolishing) is a cellulase treatment to improve the fabric quality, often done after heavy processing where pills are raised. In the enzymatic depilling of cellulosic fabrics such as cotton and Lyocell, these pills and fuzz are enzymatically removed (Cavaco-Paulo *et al.*, 1998). As in enzymatic ageing, EG or EG-enriched mixtures are most effective. Cellulase enzymes will weaken the fibres protruding from the surface by degradation, preferably of the amorphous structure of the fibre. The enzyme-weakened fibres are sensitive to shear forces and upon application of sufficient shear the fibre will break from the surface (Cavaco-Paulo *et al.*, 1996, 1997; Lenting and Warmoeskerken, 2001a). This results in:

- improved pilling resistance;
- brighter colours;
- cleaner surface;
- improved drapeability and increased softness;
- reduction in the amount of dead and immature cotton.

Enzymatic depilling is preferably carried out after bleaching the fabric, but can be carried out after any wet textile pretreatment step, after proper and complete desizing. Enzyme treatment after dyeing can result in partial dye removal and thus colour change depending on the dye used. An enzymatic depilling process requires equipment with sufficient shear forces and mixing such as a jet (see Fig. 4.2) or a winch (Cortez *et al.*, 2001).

Today's commercially available continuous equipment does not produce enough shear forces and mixing for enzymatic depilling. Typical process conditions for an industrial depilling process are pH 4.5–6.0, temperature 45–65°C, liquid ratio 3:1–20:1 and an incubation time of 15–60 minutes. The incubation time depends on the type of machine, the liquid ratio, the fabric and the desired effect. As in the denim ageing process, the enzyme dosage depends on the type, density, porosity and hydrophilicity of the fabric and the desired effect, and is a function of treatment time, pH, temperature, liquid ratio, auxiliary chemicals and the type of equipment (shear force and mixing). In general, non-ionic surfactants and dispersing agents are recommended and will enhance the overall performance. As in denim ageing, the cellulase enzymes need to be inactivated after the desired effect is obtained. Insufficient inactivation will result in extended degradation of



4.2 Jet, typically used for biopolishing processes.

cellulose and therefore an undesirable strength loss and weight reduction. Different suitable inactivation procedures can be found in the section on enzymatic ageing previously.

4.3.3 Scouring of cotton

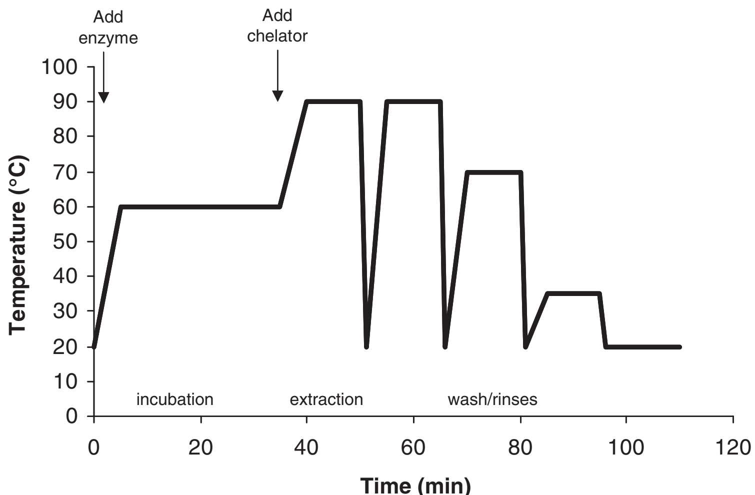
Before grey cotton fabric can be dyed and finished it has to be treated in order to make it hydrophilic and to remove the primary cell wall (see preceding chapters). In conventional cotton scouring processes high temperatures (90–100°C) and high concentrations of NaOH (approx. 1 mol/L) are used to remove the primary cell wall (pectin, protein, organic acids) and hydrophobic components from the cuticle (waxes and fats) in a non-specific way to make the fibre hydrophilic. Owing to the high NaOH concentration, extensive washing and rinsing is required, causing increased water consumption. The use of high concentrations of NaOH also requires the neutralisation of the wastewater, which requires additional chemicals. It is obvious that this process needs to be improved considerably to meet today's energy and environmental demands. Much research has been directed to replace this process with an enzymatic one (see for example: Agrawal *et al.*, 2002; Buchert *et al.*, 2000; Buschle-Diller *et al.*, 1998; Csiszár *et al.*, 2001; Etters, 1999; Hartzell and Hsieh, 1998; Lenting *et al.*, 2002; Li and Hardin, 1998; Tzanov *et al.*, 2001; Yachmenev *et al.*, 2001). The potential to degrade and remove the undesired components from the cotton

fibres of different enzymes, such as pectinases, cellulases and lipases, as well as different process conditions, have been investigated.

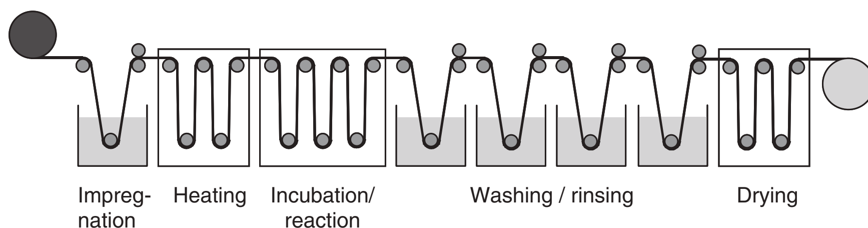
Novozymes, Bayer and Dexter Chemical Corporation have introduced an enzymatic alternative for scouring woven and knitted cotton fabrics in the textile industry on the basis of an alkaline pectinase (EC 4.2.2.2) produced by a genetically modified *Bacillus* strain. On an industrial scale, the bioscouring process using alkaline pectinases has been performed successfully in batch (pad-batch) and continuous (open width) processes (Lange and Henderson, 2000; Lange, 2000), and integrated with desizing in batch (pad-batch) and continuous (J-box) processes (Waddell, 2002). The idea is that pectin acts as a sort of cement or matrix that stabilises the primary cell wall of the cotton fibres. During incubation the enzymes will degrade pectin, thereby destabilising the structure in the outer layers. The weakened outer layers can be removed in a subsequent wash process. The bioscouring process results in textiles being softer than those scoured in the conventional NaOH process, however the degree of whiteness is often less and the process is not suitable for removing seed coat fragments and motes adequately.

A typical time–temperature profile for the enzymatic scouring of woven and knitted cotton fabrics in a jet machine is shown in Fig. 4.3 (data from Novozymes, Bayer, Lange and Henderson, 2000, Lange 2000 and Waddell, 2002). These conditions are applicable to most exhaustion machinery. The liquor ratio is 8:1 and the enzyme dosage should be between 0.5 and 1.0%.

Wetting agents and non-ionic surfactants should be added together with the enzyme to enhance enzyme penetration and adsorption, fibre swelling



4.3 Typical time–temperature profile for a jet bioscouring process.



4.4 Process scheme for a (semi-)continuous pad-steam bioscouring process.

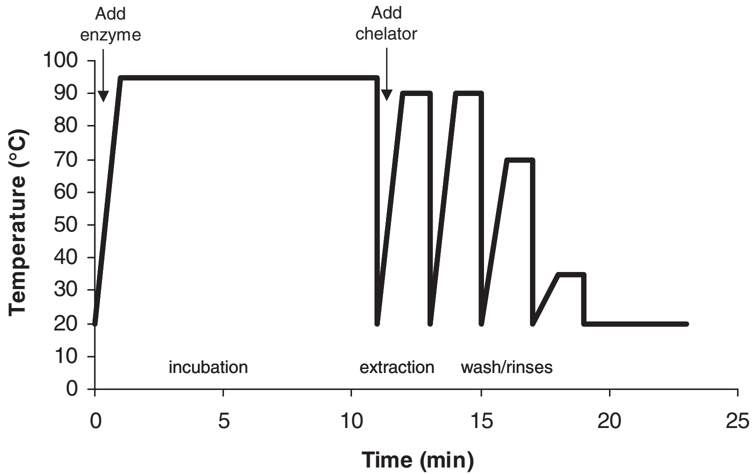
and the removal of waxes. A buffer is needed, e.g. a phosphate or citrate buffer, to maintain the pH between 7 and 9.5 (optimal pH 8.5–9.0). Combined with desizing the pH should not exceed 7.5–8.0 (see Section 4.3.1). The Ca^{2+} concentration is an important parameter in the enzymatic process, its presence slowing down the degradation of pectin but stabilising the enzyme. Therefore the addition of strong chelators is recommended only for the extraction and washing/rinsing phase. The weakened outer layers can be removed in the washing and rinsing process at a temperature above the melting point of the waxes (75–95°C), in the presence of chelators, emulsifiers and wetting agents. The process conditions for a pad-batch system are more or less identical, except that the incubation phase needs to be 1–4 hours at 60°C and 12–16 hours at 25°C.

In (semi-)continuous pad-steam machinery much shorter processing times can be realised (Lange and Henderson, 2000). The process scheme and a typical time–temperature profile for a continuous enzymatic scouring process are shown in Fig. 4.4 and 4.5, respectively. The processing conditions resemble those of the batch process described above except that the temperature during the incubation phase might be raised up to 95°C. Lower temperatures, but above 55°C, might be applied as well in this process. This is because the fabric being introduced does not reach full the temperature immediately and thus the enzyme has time to degrade the pectin before being deactivated by the high temperature. Other modifications to increase the process speed are a short hot-water treatment (Hartzell and Hsieh, 1998; Agrawal *et al.*, 2002) or a rinse at 50°C (Waddell, 2002) prior to the bioscouring process.

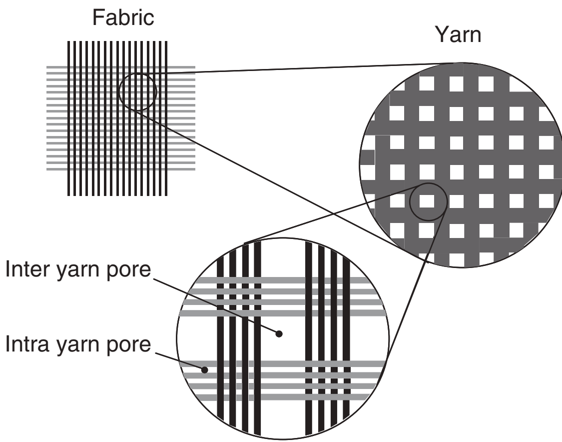
4.4 Mass transfer in textile materials

4.4.1 The structure of textile materials

Textile materials can have different structures such as woven and knitted fabrics, and non-wovens. In the context of this chapter we limit ourselves

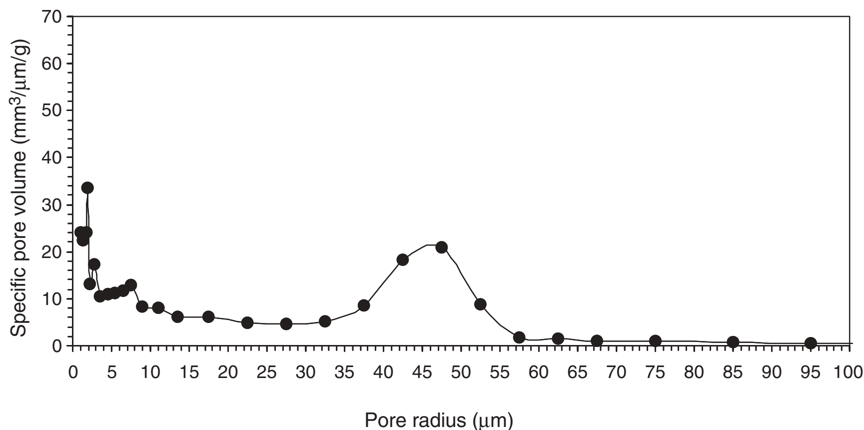


4.5 Typical time–temperature profile for a pad-steam bioscouring process.



4.6 Structure of a woven textile material (schematically).

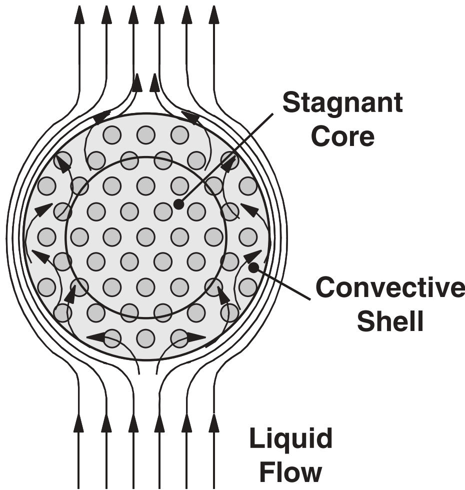
to discussing woven textiles. To make a woven textile, fibres are spun into yarns and yarns are woven into fabrics. This means that textiles can be seen as a porous slab with two kind of pores, pores between the fibres, the intra-yarn pores and pores between the yarns, the inter-yarn pores. This is schematically drawn in Fig. 4.6. This is why we say that woven textiles have a dual porosity. Figure 4.7 shows an example of the specific pore volume distribution in a cotton fabric, measured by a TRI-autoporosimeter (Textile



4.7 Pore size distribution of a woven cotton fabric.

Research Institute). From this figure it is clear that the cloth contains small pores, in the order of 2 μm, the intra-yarn pores, and larger pores in the order of 47 μm, the inter-yarn pores. Many investigators have studied and measured the pore size distributions in textile materials with respect to flow phenomena in textiles (Van den Brekel, 1987; Van den Brekel and de Jong, 1988; Gooijer, 1998).

The migration of the enzyme molecules into the intra-yarn pores is necessary for good enzymatic treatment of the fibres within a yarn. This can be achieved by flowing an enzyme solution through the fabric. However, since the flow resistance in the intra-yarn pores is much higher than the resistance in the inter-yarn pores, the bulk of the liquid will flow along the yarns instead of through the yarns. This was found by Van den Brekel and later confirmed by Gooijer. In Fig. 4.8 the flow pattern of a liquid flowing along a yarn is drawn schematically. Based on this Warmoeskerken and Boom (1999) introduced the concept of a stagnant core and a convective shell. The stagnant core of the yarn is the area in which there is no flow at all. The convective shell is the outer area of the yarn in which the flow penetrates to some extent. The transfer processes in the stagnant core are based on molecular diffusion while the transport processes in the outer convective shell are driven by convective diffusion. Since convective diffusion is much faster than molecular diffusion, the rate of mass transfer in the yarn will be determined by the size of the stagnant core. This means that the migration time of enzymes into the intra yarn pores is determined by molecular diffusion in the stagnant core, which is a relatively slow process.



4.8 Liquid flow around and through a textile yarn. The dots represent the fibres in the yarn.

4.4.2 Diffusion of enzymes in a yarn

The diffusion time of enzymes in a yarn can be calculated by applying the theory for molecular diffusion that can be found in textbooks by Crank (1956) and Carslaw and Jaeger (1989). In the following approach, the direction of diffusion can be from the outside area to the centre of the yarn or from the yarn centre to the outside area. For the diffusion model we adapted the diffusion in a cylinder. The general equation describing this diffusion process in cylindrical coordinates is:

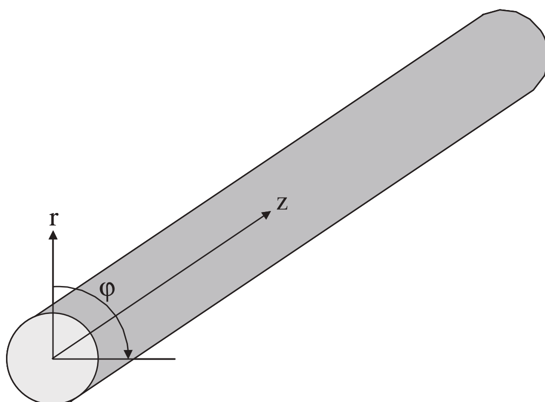
$$\frac{\partial C}{\partial t} = D\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C}{\partial r}\right) + \frac{1}{r^2}\frac{\partial^2 C}{\partial \varphi^2} + \frac{\partial^2 C}{\partial z^2}\right] \tag{4.1}$$

in which t is the time, C is the time- and place-dependent concentration of the enzymes and r, φ, and z are the axes along which the diffusion process proceeds, see Fig. 4.9.

D is the diffusion coefficient in m^2/s of the diffusing enzyme. If only diffusion in the radial direction is considered, equation [4.1] reduces to:

$$\frac{\partial C}{\partial t} = \frac{D}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C}{\partial r}\right) \tag{4.2}$$

This equation can be solved for different initial and boundary conditions. In the current case we consider the situation in which the enzymes diffuse from a bulk solution into the yarns and we assume that the enzyme concentration in the bulk remains constant because the volume of the liquid



4.9 Schematic representation of the coordinate system used to model diffusion in a yarn.

bulk is much higher than the volume of the intra-yarn pores. This means that the concentration of the enzymes at the outer surface of the yarn is constant and equal to the bulk concentration. The second assumption is that at the start of the diffusion process no enzymes are present in the yarn. So the initial and boundary conditions can be written as:

$$t = 0 \quad 0 \le r \le \frac{1}{2} d_{\text{yarn}} \quad C = 0 \qquad [4.3]$$

$$t > 0 \quad r = \frac{1}{2} d_{\text{yarn}} \quad C = C_{\text{bulk}}$$

with C_{bulk} being the enzyme concentration in the bulk and d_{yarn} the yarn diameter.

The solution of equation [4.2] with the initial and boundary conditions according to equation [4.3] is a Bessel function and reads:

$$E = \frac{\overline{C}}{C_{\text{bulk}}} = 1 - \sum_{n=1}^{n=\infty} \left[\frac{4}{\mu_n^2} \exp(-4\mu_n^2 F_0) \right] \qquad [4.4]$$

with $\overline{C}$ being the average enzyme concentration in the yarn and:

$\mu_1 = 2.4048$
$\mu_2 = 5.5201$
$\mu_3 = 8.6537$
$\mu_4 = 11.7953$
$\mu_5 = \mu_4 + \pi$
. . .
. . .
. . .
$\mu_n = \mu_{n-1} + \pi$

F_0 is the dimensionless Fourier number and is defined as:

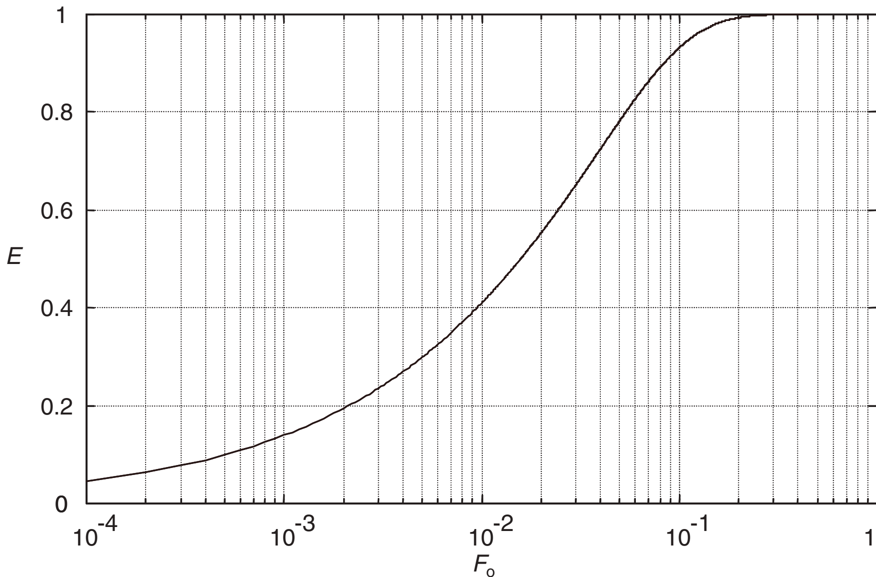
$$F_0 = \frac{Dt}{d_{\text{yarn}}^2} \tag{4.5}$$

and the mean enzyme concentration in the bulk is defined as:

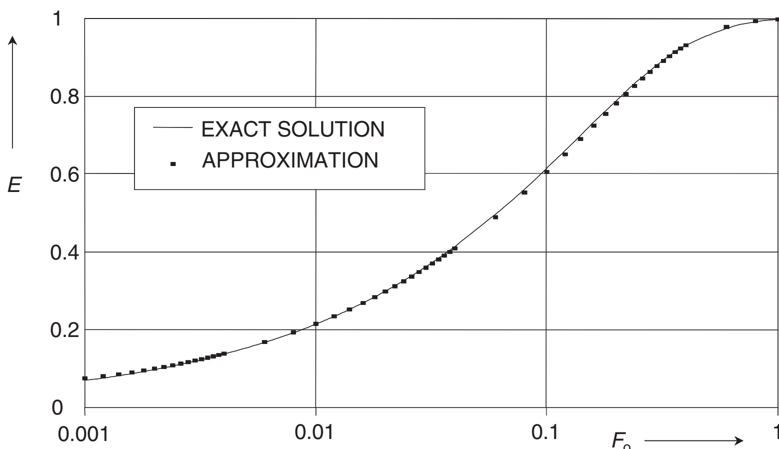
$$\overline{C} = \frac{2}{d_{\text{yarn}}} \int_0^{\frac{1}{2}d_{yarn}} C\mathrm{d}r \tag{4.6}$$

The F_0 number represents the ratio of the process time t and the diffusion time d_{yarn}/D. E in equation [4.4] is the dimensionless mean concentration of the enzymes in the yarns. This is also called the efficiency of the diffusion process. At $t = 0$, when the mean enzyme concentration in the yarn is zero, the value of E is 0 as well, and after an infinitely long time when the mean enzyme concentration in the yarn equals the bulk concentration, E has the value of 1. Figure 4.10 shows the calculated result of equation [4.4].

In the calculations the value of n was taken as 25. However, the solution of the diffusion equation in the form of Bessel functions is not very easy to use. To overcome that problem the approximation method of Etters (1980) is very useful. He fitted the exact solution of equation [4.1] by:



4.10 The exact solution of the diffusion problem (equation [4.4]).



4.11 The approximate solution from Etters of the diffusion problem, and the exact solution.

$$E = \frac{\overline{C}}{C_{\text{bulk}}} = \left[1 - e^{-a(4F_0)^b}\right]^c \qquad [4.7]$$

In our case where there is no diffusion boundary layer between the bulk solution and the yarn, see the boundary conditions in equation [4.3], the values for the constants are $a = 5.530$, $b = 1.0279$ and $c = 0.3341$. Figure 4.11 shows the calculated results of E as a function of F_0 according to the exact solution, equation [4.4], and according to the approximation formula, equation [4.7]. From this figure it can be concluded that the approximation formula of Etters leads to good results. With this formula the diffusion time can be calculated if the diffusion coefficient of the enzymes is known.

4.4.3 The diffusivity of enzymes

Since we have worked with dimensionless numbers like the Fourier number, until now we did not need the value for the diffusion coefficient of enzyme molecules. If we want to make calculations for the diffusion time of enzymes in yarns we need to find values for the diffusion coefficients. Measurement of the diffusion coefficient is rather complicated. Most methods, mentioned by Van Holde (1971) and by Sun (1994), are based on the application of Fick's first law for diffusion. This law reads:

$$V\frac{\mathrm{d}C}{\mathrm{d}t} = DA\Delta C \qquad [4.8]$$

in which V is the liquid volume in m^3 in which the diffusion process proceeds, A is the surface area in m^2 through which the molecules diffuse, D is the diffusion coefficient in m^2/s, C is the concentration of the diffusing component in kg/m^3 and ΔC is the concentration difference. The most common procedure is to separate two liquids from each other by a membrane. In one liquid the diffusing component is absent at time $t = 0$. In the other liquid the diffusion of the component is followed by optical methods such as Schlieren and refraction.

It is also possible to calculate the diffusion coefficient; we can apply the Stokes–Einstein relation (Bird *et al.*, 1960). This relation reads:

$$D = \frac{k_B T}{3\pi \eta_L d_M} \qquad [4.9]$$

in which D is the diffusion coefficient in m^2/s, k_B is the Boltzman constant in J/K, T the temperature in K, η_L the dynamic viscosity of the liquid in which the molecules diffuse in Pa.s, and d_M the diameter of the diffusing molecule in m. Assuming the enzyme molecule is a sphere, its diameter can be calculated by:

$$d_M = \sqrt[3]{\frac{6 M_W 10^{-3}}{N_{AV} \rho_M \pi}} \qquad [4.10]$$

in which M_W is the molecular weight of the enzymes in kg/kmol, N_{AV} is the Avogadro number in mol^{-1} and ρ_M is the density of the molecule in kg/m^3.

From equations [4.9] and [4.10] follows the relation between the molecular weight of the enzyme molecule and its diffusivity in a liquid:

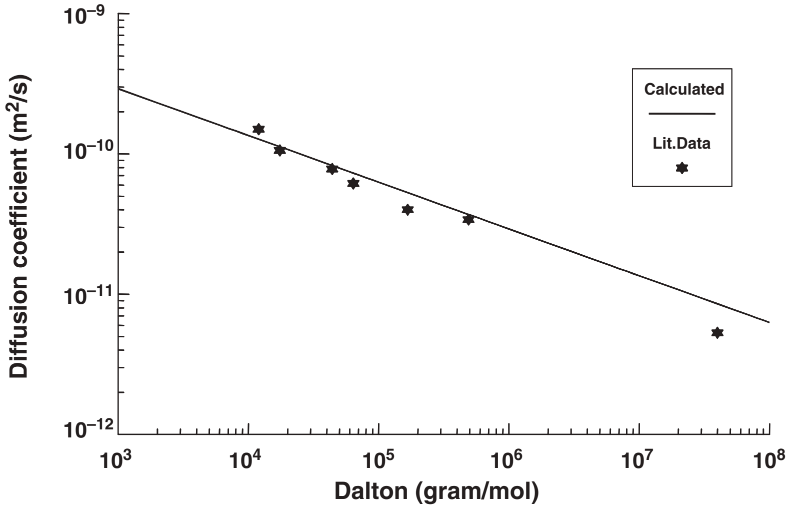
$$D = \frac{k_B T}{3\pi \eta_L \sqrt[3]{\frac{6 M_W 10^{-3}}{N_{AV} \rho_M \pi}}} \qquad [4.11]$$

Figure 4.12 shows some results of this equation. In this figure the measured data of Daniels and Alberty (1975) for the diffusion coefficient of large protein molecules in water are compared with the calculated values according to equation [4.11]. From the figure it can be concluded that equation [4.11] gives reasonable to good results for the diffusion coefficients of enzymes.

Since we are focusing on the diffusion of enzymes in textiles we have to take account of the porosity of the system. The effect of the porosity on the diffusion coefficient can be expressed as:

$$D_{porous} = D\varepsilon \qquad [4.12]$$

in which D_{porous} is the effective diffusion coefficient and ε the porosity of the system. So the diffusion coefficient in porous systems is smaller than that in homogeneous systems. This is because in the yarn the fibres decrease



4.12 Calculated and measured diffusion coefficients (experimental data from Daniels and Alberty, 1975).

the free area through which the enzymes diffuse. Another aspect that affects diffusion in porous systems is the tortuosity. In a yarn the enzyme molecules cannot diffuse via the shortest path, that is via the radius to the centre, because the fibres obstruct the straight path to the centre. So in reality the enzymes have to diffuse through a labyrinth of fibres, which makes the actual diffusion path longer. This effect is expressed in the tortuosity β, which gives the ratio between the actual path length for diffusion and the free path length. For a yarn consisting of fibres, the tortuosity β has a value of 2. This means that the actual diffusion length in the radial direction of the yarn is twice the radius of the yarn. From the diffusion equation it can be derived that the diffusion coefficient in a tortuous system is:

$$D_{\text{tortuous}} = \frac{D}{\beta^2} \qquad [4.13]$$

If we now combine equations [4.12] and [4.13] we find an expression for the effective diffusion coefficient D_{eff} that includes the effect of porosity as well as that of tortuosity:

$$D_{\text{eff}} = \frac{D\varepsilon}{\beta^2} \qquad [4.14]$$

From this equation we can calculate that the diffusion coefficient of enzymes in a yarn with a porosity $\varepsilon = 0.5$ and a tortuosity $\beta = 2$ is a factor of 0.125 smaller than the diffusion coefficient in a homogeneous system.

In an example we will now calculate the diffusion time of enzymes in a yarn. Suppose we have a yarn with a diameter of 0.5 mm, a porosity of 0.4 and a tortuosity of 2. In Fig. 4.10 we see that the diffusion process is more or less completed when the F_o number has a value of 1. With equation [4.5] we can derive:

$$t = \frac{F_o d_{yarn}^2}{D_{eff}} \quad [4.15]$$

or with the values for F_o and d_{yarn} chosen above:

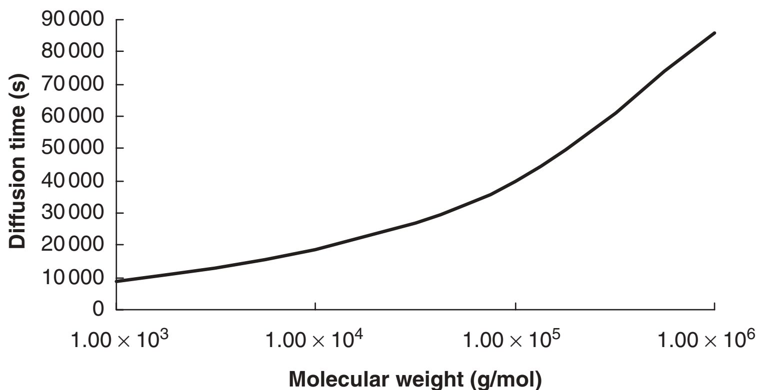
$$t = \frac{2.5 \times 10^{-7}}{D_{eff}} \quad [4.16]$$

The effective diffusion coefficient as function of the molecular weight of enzyme molecules can be calculated by equations [4.11] and [4.14]. We have done this for the following values of the parameters:

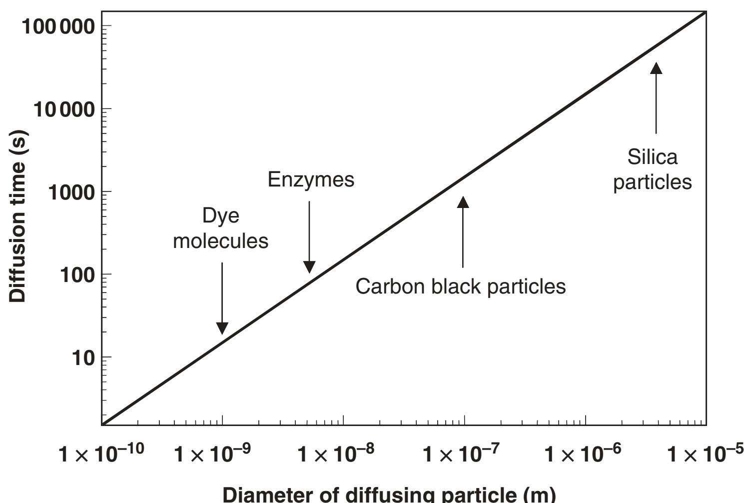
ρ_M	=	1000	kg/m^3
μ_L	=	10^{-3}	Pa.s
T	=	293	K
N_{AV}	=	6×10^{23}	mol^{-1}
k_B	=	1.38×10^{-23}	J/K

Figure 4.13 shows the results of the calculations. From this figure it is clear that the time to complete the diffusion process is already in the order of 5 hours for an enzyme with a molecular weight of 20 000 g/mol. This process time is not available in continuous textile treatment processes.

We have repeated the calculations for different materials such as dye molecules, enzymes, carbon black particles and silica particles. In each



4.13 Time needed to complete the diffusion process.



4.14 Time needed to remove 90% of particles from a yarn by diffusion as a function of the particle diameter.

case we calculated the time needed to complete 90% of the diffusion process in a yarn. In the case $F_o = 0.1$, see Fig. 4.10, the results have been drawn in Fig. 4.14 in which the calculated diffusion time is plotted against the diameter of the diffusing particle. From the figure it is clear that the diffusion times are lower for lower values of F_o, although they are still high compared to process times. Figure 4.14 also shows that the diffusion time increases non-linearly with the diameter of the diffusing particle.

Thus, taking into account the typical porous structure of textile materials and the relatively large size of the enzyme molecules, the physical transport of the molecules is often a rate-limiting step in enzymatic textile treatment processes. The only way to lower the transport time is by decreasing the stagnant core, thus creating convective flow in the intra-yarn pores of the fabric.

It has to be mentioned that this is only the case in so-called wet-to-wet applications. In wet-to-dry applications the enzyme solution can penetrate into the pores by the so-called wicking effect. This is the result of capillary forces which allow the enzyme solution to penetrate directly into the pores of the yarn. Thus it is clear that the transport of enzymes into the textile material is much faster in the case of wet-to-dry applications than in wet-to-wet systems owing to wetting and wicking. Wetting and wicking of textile materials is a subject beyond the scope of this chapter.

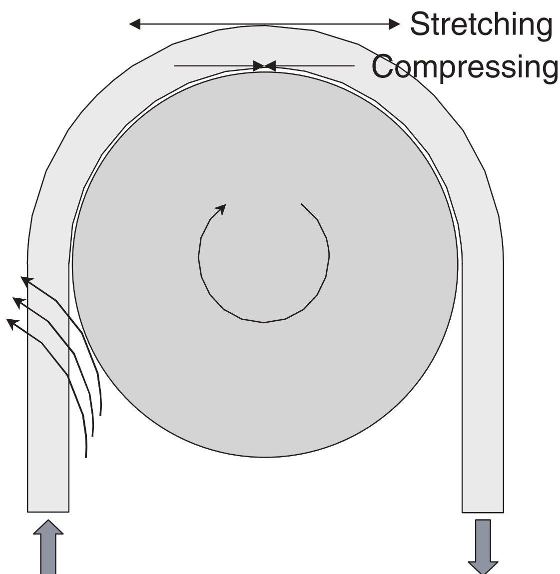
4.5 Process intensification: enhancement of mass transfer in textile materials

Process intensification is important when introducing new processes into the textile industry. Process intensification will not only result in more efficient and economically feasible processes, it also offers possibilities for production on demand because of a dramatic decrease in the residence time. From the data presented in Section 4.3 it is clear that the residence time in enzymatic textile pretreatment and finishing processes, like that in conventional textile processes is still relatively long. In Section 4.4 it was shown that the activity of enzymes in textile treatment processes can be limited to a large extend by their slow diffusion into the pores of a yarn. Decreasing the diffusional core or the stagnant core in the yarn can enhance this transport rate. In other words by creating flow in the intra-yarn pores, the rate of the mass transfer process is then determined by convection which is always much faster than diffusion. In the present section the possibilities of enhancing mass transfer through the deformation of textile materials and the application of ultrasound are discussed as tools for intensifying enzymatic wet textile processes.

4.5.1 Deformation of textile materials

The most common way to enhance mass transfer in the intra-yarn pores is to deform the porous matrix of the textile material. When a force is applied to the textile the pores become smaller resulting in a flow of the pore liquid to the treatment bath. If thereafter the force is released, the textile system relaxes, the pores recover their original shape and liquid flows from the bath into the pores. This so-called squeezing effect can be obtained in different ways. In an open-width process this phenomenon occurs at the moment the textile passes a roller. This is drawn schematically in Fig. 4.15.

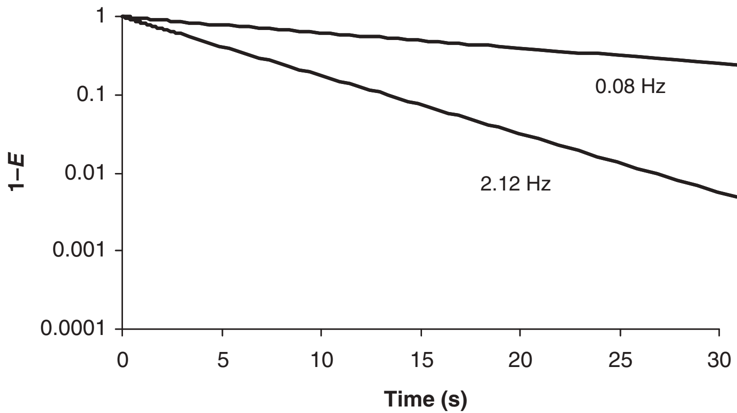
On the side where the textile is attached to the roller the textile is deformed by compression forces. On the outside the textile is deformed by stretching forces. Although this phenomenon is well known, quantitative data about the internal flow in the textile pores that is generated by this mechanism is not known. At the point at which the fabric contacts the roller, the liquid is forced through the textile as indicated in Fig. 4.15. Also no quantitative data are available about this mechanism. This is why the design of this kind of process equipment is often based on trial and error. As far as we know, only Van der Donck *et al.* (1998) have done some work on the influence of squeezing on mass transfer. Farber and co-workers (Farber and Dahmen, 1998; Farber *et al.* 1999), for example, used advanced computational fluid dynamics (CFD) to describe mass transfer in open width equipment, but despite the quality of their model and the outcome of their



4.15 Deformation of a textile on a roller.

simulations, the textile material is still described as a homogeneous permeable rigid structure instead of a deformable biporous structure. Van der Donck *et al*. studied the squeezing effect in yarns when they are stretched and concluded that this mechanism contributes to a large extent to the mass transfer rate in textile yarns.

Van der Donck *et al*. reported that deforming yarns by placing a fabric in a pulsating flow or repeated deformation through mechanical elongation of the yarns improved mass transport compared with diffusion alone. When a yarn is elongated a quantity of liquid, as well as the enzymes or chemicals in that liquid, is squeezed out of the yarn. Van der Donck measured the increase of conductivity caused by the release of magnesium sulphate with time from a cotton yarn impregnated with magnesium sulphate. The magnesium sulphate was squeezed out of the yarn by the repeated elongation of that yarn. In practice this is realised at the rollers of open-width equipment or through tumbling the cloth in domestic laundry machines. To describe the phenomena that are observed, Van der Donck used the dimensionless Fourier number, which gave a qualitative logarithmic relation between the soil release, the Fourier number and the additional salt removed. However, Etters (1980) proposed a mathematically simple empirical equation that matches the exact solution to describe diffusion-controlled mass transport in yarns. Equation [4.7] has been extended in such



4.16 Calculated relative soil removal as function of time using the modified equation of Etters for different deformation frequencies. ($D_{eff} = 4.8 \times 10^{-10}\,m^2/s$, $d_{yarn} = 3.5 \times 10^{-4}\,m$, $\varepsilon_{intra\text{-}yarn} = 0.5$, $\varepsilon_{deformation} = 0.03$, $a = 2.440$, $b = 1.045$, $c = 0.863$).
Van der Donck *et al.* used a frequency of 0.08 and 2.12 in their experiments.

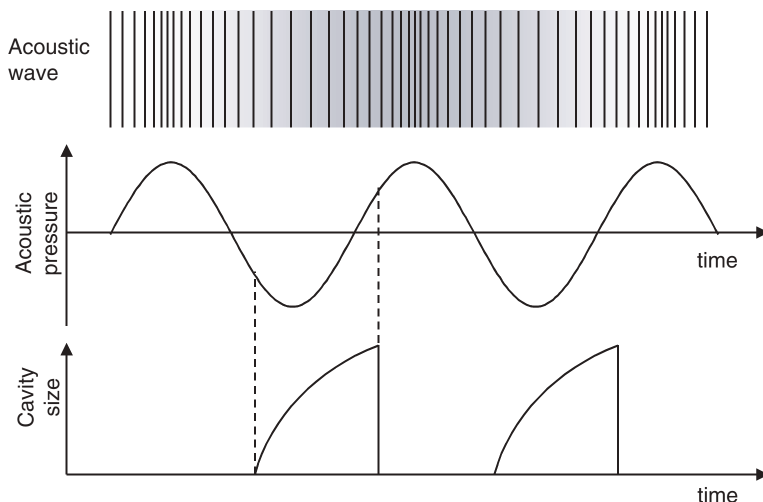
a way that it is possible to describe the influence of the deformation of the yarn on mass transport:

$$1 - E = \left[1 - \exp\left(-a\left(\frac{4D_{eff}t}{d_{yarn}^2}\right)^b\right)\right]^c \left(\frac{\varepsilon_{intra\text{-}yarn} - \varepsilon_{deformation}}{\varepsilon_{intra\text{-}yarn}}\right)^{ft} \quad [4.17]$$

where $\varepsilon_{intra\text{-}yarn}$ is the porosity of the yarn, $\varepsilon_{deformation}$ is the volume fraction squeezed out of the yarn during deformation and f is the frequency of the deformation. With this modified equation of Etters we are able to describe increased mass transport caused by stretching the yarns. In domestic laundry processes the deformation of the plug and therefore of the yarns is related to the rotation velocity of the drum. Thus, in industrial open-width equipment and in a domestic laundry processes a deformation frequency between 0 and 2.5 Hz seems realistic. In Fig. 4.16 the results are shown using the modified equation from Etters. Different constants for a, b and c in Equation [4.17] were used to correct for the low amount of mixing in the experiments of Van der Donck *et al.* The calculated soil removal time compares very well with the results described by Van der Donck.

4.5.2 Ultrasound-enhanced mass transfer

Another way of enhancing the mass transfer is the application of ultrasonic waves. Ultrasound as a means of intensification of wet textile processes has been attempted by several researchers (e.g. McCall *et al.*, 1998; Thakore,



4.17 Some characteristics of an ultrasonic wave.

1990; Yachmenev *et al.*, 1998, 1999, 2001; Rathi *et al.* 1997). In spite of encouraging results in laboratory scale studies, ultrasound-assisted wet textile processes have not yet been implemented on an industrial scale. Two major factors that have contributed to this are lack of precise knowledge about the physical mechanism of ultrasonic mass transfer enhancement in textiles and the inherent drawbacks of ultrasonic processors, such as directional sensitivity, erosion of sonicator surface and the non-uniform volumetric energy dissipation.

Ultrasound is a longitudinal pressure wave in the frequency range above 25 kHz (see Fig. 4.17). As the sound wave passes through water in the form of compression and rarefaction cycles the average distance between the water molecules varies. If the pressure amplitude of the sound is sufficiently large, the distance between the adjacent molecules can exceed the critical molecular distance during the rarefaction cycle. At that moment a new liquid surface is created in the form of voids. This phenomenon is called acoustic cavitation and is drawn schematically in Fig. 4.17.

The theoretical pressure amplitude that causes cavitation in water is approximately 1500 bar. However, in practice acoustic cavitation occurs at a far lower pressure amplitude, less then 5 bar. This is due to the presence of weak spots in the liquid in the form of tiny microbubbles that lower the tensile strength of the liquid. Once formed, the bubbles can redisolve into the liquid; they may float away, or, depending on their size, they may grow and shrink in phase with the oscillating ultrasonic field. This process of growing and recompressing bubbles is called stable cavitation. If the sound

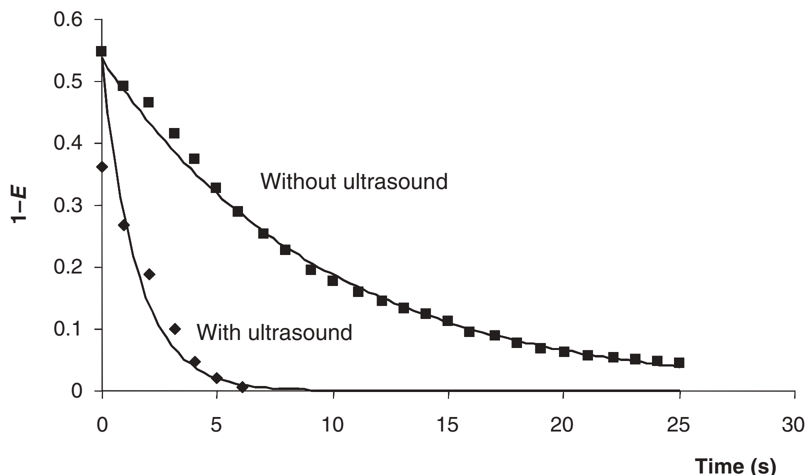
field is sufficiently intense, bubbles of a specific initial size can grow so quickly and acquire such momentum that the compression wave that immediately follows the rarefaction phase is no longer able to stop the bubbles growing. Once out of phase with the ultrasonic field, however, the bubbles are no longer stable. The pressure within the bubble is not high enough to sustain the size of the bubble and, driven by the next compression wave, the bubbles implode. This latter process is called transient cavitation.

In liquids the collapsing bubbles remain spherical because the ultrasonic waves are uniform. However, if a transient acoustic bubble collapses near a solid boundary, the bubble will implode asymmetrically, generating jets of liquid directed towards the surface of the solid boundary. The microjets resulting from collapsing bubbles at a solid boundary account for the well-known cleaning effect of ultrasonic waves. This acoustic cavitation process has been described by many authors, for example Neppiras (1980), Apfel (1981) and Suslick (1988).

This area of power ultrasound is what we have called sonomechanics, making a clear distinction from sonochemistry. The latter is the application of power ultrasound to speed up chemical reactions. The idea is that if an acoustic cavity collapses adiabatically the temperature of the very small volume of liquid involved must rise several thousands of degrees K enhancing the rate of chemical reaction. A lot of literature about this subject is available, for example Mason and Lorimer (1989) and Mason (1990). Ultrasound has also been found to have an effect on enzymatic reactions (Warmoeskerken *et al.*, 1994). However it is not clear whether the observed enhancement in the enzymatic reaction rate is due to the temperature effect mentioned above or to a more intrinsic effect, such as unfolding the enzyme molecule so that the reactive site becomes more accessible to the substrate. More fundamental research is needed in this area to clarify the mechanism and to develop a process in which the enzymatic reactions are boosted by ultrasound.

Here we restrict ourselves to sonomechanics, the application of ultrasound to enhance mass transfer in textile materials. Much research can also be found in this area (Yachmenev *et al.* 1998, 1999, 2001; Moholkar 2002; Moholkar and Warmoeskerken, 2000, 2001, 2002; Moholkar and Pandit, 2001; Moholkar *et al.*, 2000, 2002; Warmoeskerken *et al.*, 2002).

We have found that ultrasound can speed up mass transfer in textile materials. Figure 4.18 shows the results of a typical experiment (Warmoeskerken, 2002). A textile cloth was impregnated with a salt that was then washed out in water. The release of salt from the textile with time was followed by conductivity measurements in the bath. One experiment was performed without ultrasound and one with ultrasound. The results of these experiments are shown in Fig. 4.18 where $(1 - E)$, representing the fraction of salt that is still on the cloth, is plotted against the process time.

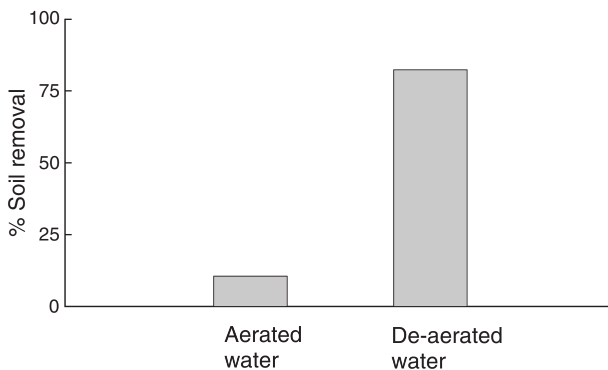


4.18 Experimental salt-rinsing results with and without the application of ultrasound.

From Fig. 4.18 it is clear that in the case where ultrasound is applied salt release is much faster than in the case without ultrasound.

The mechanism of this phenomenon involves the formation of transient acoustic cavities in the close vicinity of the textile surface. These asymmetrically collapsing cavities create locally microscale liquid jets that are directed towards the substrate surface and penetrate deeply into the pores of the textile. It can be argued, with respect to the stagnant core–convective shell model, that ultrasound decreases the stagnant core in the yarn, resulting in an enhanced mass transfer rate in the yarn.

The development of a more efficient textile treatment or enzymatic textile treatment process based on these findings is not simple. An ultrasonic system is quite complex and the performance is dependent on all the system parameters, for example the size of the system, the properties of the ultrasonic equipment, the frequency and power of the ultrasonic wave and the composition of the liquid. A very important parameter seems to be the presence of air in the water. The ultrasonic wave, while traveling from the transducer to the substrate will create a lot of acoustic cavities in the bulk liquid between the transducer and the substrate. However, since the transient cavities are only required at the substrate surface, the formation of cavities in the bulk liquid is in fact only a waste of energy. In a deaerated system there are no energy losses during the time that the ultrasonic wave travels from the transducer to the substrate and all the ultrasonic energy is applied to the formation of transient cavities at the substrate surface. Therefore some air pockets have to be available at the substrate surface. In



4.19 Soil removal from Empa 101 with the application of ultrasound in aerated and de-aerated water.

practice there is always sufficient air present in the textile material for the intended ultrasonic effect.

Figure 4.19 shows some results of cleaning an Empa 101 test cloth (Moholkar, 2002). This is a test monitor that is used to study the performance of laundry systems and is cotton impregnated with a mixture of olive oil and carbon black particles. Figure 4.19 shows that in the deaerated case the performance of the ultrasonic wave, in terms of soil removal, is more than seven times better than in the aerated case. Although these are very promising results, a lot of research is still needed to translate the current knowledge about ultrasonically boosted wet textile treatment processes to an operational full-scale process.

4.6 Mass transfer and diffusion limitation in immobilised enzyme systems

In some industrial textile processes enzymes or microorganisms are immobilised; for example to prevent them from flushing out of the (bio)reactor during a continuous operation such as the decolourisation of textile effluents (Oxspring *et al.*, 1996; Mielgo *et al.*, 2001, 2002). To immobilise biocatalysts (enzymes or complete microorganisms) several methods are available such as entrapment in a porous support or attachment of the biocatalyst to a surface (for more details see e.g. Shuler and Kargi, 2002 and van't Riet and Tramper, 1991). In general the most important advantages of immobilising biocatalysts are:

- the possibility of reuse;
- the possibility of continuous operation;
- a more stable or active enzyme (different microenvironment);
- elimination of enzyme recovery and purification processes.

However, immobilisation of enzymes or microorganisms can cause mass transfer limitations compared to free enzymes or microorganims. Diffusional resistance may be observed at different levels depending on the nature of the support, the turbulence in the reactor (the Reynolds number), the particle size and the distribution and concentration of the enzyme over the particle. Simply decreasing the particle size and increasing the porosity to eliminate or to reduce mass transfer problems is not always desirable for optimal reactor performance (Van 't Riet and Tramper, 1991).

Whether diffusion resistance in a heterogeneous biocatalyst (the particle, or 'bead' with immobilised enzymes or microorganisms) has a significant effect on the overall reaction rate depends on the ratio of the enzymatic reaction rate and the diffusion rate, which is characterised by the Damköhler number (Da).

$$Da = \frac{\text{maximum rate of reaction}}{\text{maximum rate of diffusion}} = \frac{V_{\text{max}}}{k_{\text{sl}} A' K_{\text{m}}} \quad [4.18]$$

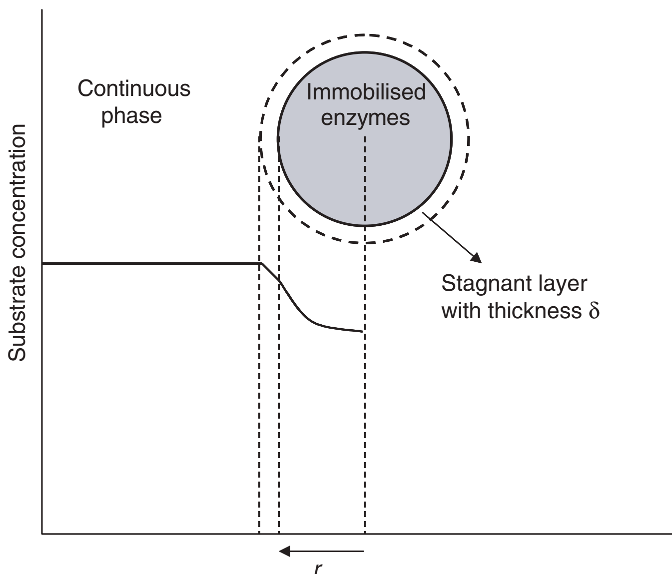
where V_{max} (mol/(s·m^3) is the maximal rate of conversion, K_{m} (mol/m^3) is the Michaelis–Menten constant, k_{sl} is the mass transfer coefficient (m/s) and A' is the specific surface area (m^{-1}). If $Da >> 1$, the diffusion rate in the heterogeneous biocatalyst is limiting; for $Da << 1$, the reaction rate is limiting compared to mass transfer by diffusion; and for $Da \approx 1$, the diffusion and reaction rates are comparable.

4.6.1 Diffusion effects in immobilised enzyme systems

Mass transfer limitation can occur in the biocatalyst particle itself, in the external layer surrounding the particle, or in both (see Fig. 4.20). In the bead, mass transfer is in principle only possible by diffusion. Concentration gradients are the driving force for diffusion. Because of the consumption of substrate by enzyme, the concentration in the particle will decrease and a concentration gradient will develop. This results in a net flow of substrate from the surface of the particle to the centre of the particle. Diffusion limitation in the particle is thus a function of, for example, the particle size, the porosity and the amount of enzyme in the particle.

A thin stagnant liquid film surrounding the particle causes mass transfer limitation by diffusion in the external layer. The thickness (δ) of that stagnant film, and thus the amount of external mass transfer limitation, depends on the flow conditions in the reactor and can be described by film theory. The same analysis of external and internal diffusion limitation holds for biofilms and microbial flocs (aggregates of cells).

In general the substrate concentration in the biocatalyst particle and in the stagnant film surrounding the particle will be lower than the concentration in the continuous or bulk phase. This means that if the biocatalyst obeys Michaelis–Menten kinetics (see the preceeding chapter), the



4.20 Substrate concentration profile in and around an immobilised enzyme particle (the continuous phase is perfectly mixed).

observed reaction rate will be lower than could be expected on the basis of the bulk concentration. Quite often, apparent or lumped kinetic constants are used to describe the kinetics of immobilised biocatalysts:

$$-r_s = -\frac{\partial C_{s,bulk}}{\partial t} = V_{max}^{app} \frac{C_{s,bulk}}{K_m^{app} + C_{s,bulk}} \quad [4.19]$$

in which r_s is the rate of substrate conversion (mol/(s·m^3)), t is the time (s), $C_{s,bulk}$ is the substrate concentration in the bulk liquid (mol/m^3), V_{max}^{app} (mol/(s·m^3)) is the apparent maximal rate of conversion and K_m^{app} (mol/m^3) is the apparent Michaelis–Menten constant. However, these apparent constants depend on the radius of the particle R_p, the effective diffusion coefficient D_{eff}, and the true reaction constants K_m and V_{max}. Therefore this approach is incorrect in principal. It is better to introduce, as in heterogeneous catalysis, an overall effectiveness factor η^{ov} (–) (Van 't Riet and Tramper, 1991):

$$-r_s = -\frac{\partial C_{s,bulk}}{\partial t} = \eta^{ov} V_{max} \frac{C_{s,bulk}}{K_m + C_{s,bulk}} \quad [4.20]$$

The overall effectiveness factor is defined as the product of the external and the internal effectiveness factor:

$$\eta^{ov} = \eta_e \eta_i \quad [4.21]$$

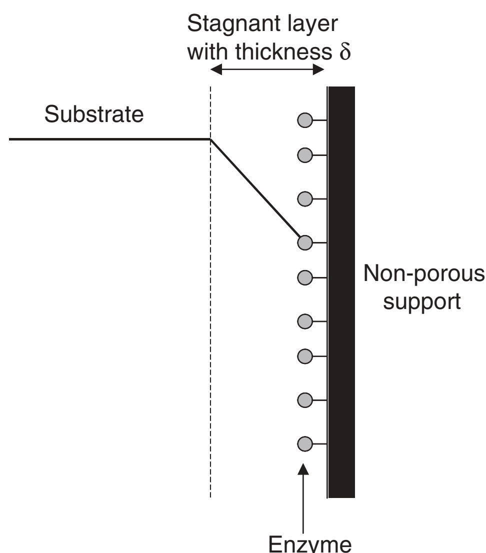
The overall effectiveness factor usually lies between 0 and 1 (Michaelis–Menten kinetics) and depends on the substrate concentration. It may sometimes be higher than unity owing to non-isothermal operation, partition effects or more complicated kinetics such as substrate inhibition kinetics.

4.6.1.1 External diffusion limitation

As explained above, because there is a thin stagnant film surrounding the biocatalyst particle, the substrate concentration is lower at the surface compared with the bulk concentration and thus the enzymes that are immobilised at the surface of the particle experience a lower substrate concentration. This is also the case for surface-bound enzymes on non-porous support materials (Fig. 4.21).

In order to correct for this an external effectiveness factor, η_e (–), is introduced, as in heterogeneous catalysis. This external effectiveness factor is defined as the ratio of the reaction rate at $C_s = C_{s,surf}$ ($C_{s,surf}$ is the substrate at the surface of the particle) and the reaction rate if external diffusion limitation is absent $C_s = C_{s,bulk}$:

$$\eta_e = \frac{-r_s(C_{s,surf})}{-r_s(C_{s,bulk})} \qquad [4.22]$$



4.21 Substrate concentration profile in a stagnant layer with enzymes bound to the surface of a non-porous support.

For Michaelis–Menten kinetics this results in:

$$\eta_e = \frac{C_{s,surf}(K_m + C_{s,bulk})}{C_{s,bulk}(K_m + C_{s,surf})} \qquad [4.23]$$

We assume that the substrate concentrations in the bulk liquid and K_m are known. This means that in order to determine the external effectiveness factor the substrate concentration at the surface should be known. In the steady state, the reaction rate at the surface is equal to the mass transfer rate J_s:

$$J_s = -r_s(C_{s,surf}) = \frac{V_{max}C_{s,surf}}{K_m + C_{s,surf}} = k_{sl}A'(C_{s,bulk} - C_{s,surf}) \qquad [4.24]$$

k_{sl} and r_s can be measured and therefore $C_{s,surf}$ and the external effectiveness factor can be calculated. Equation [4.23] can be simplified for extreme values. If the substrate concentration at the surface is much greater than K_m, equation [4.24] reduces to:

$$J_s = -r_s(C_{s,surf}) = V_{max} = k_{sl}A'(C_{s,bulk} - C_{s,surf}) \qquad [4.25]$$

Often, however, $C_{s,surf} << K_m$. Under such conditions we can consider two limiting cases. For $Da >> 1$, we can write:

$$-r_s(C_{s,surf}) = k_{sl}A'C_{s,bulk} \qquad [4.26]$$

For $Da << 1$, we can write:

$$-r_s(C_{s,surf}) = C_{s,bulk}\frac{V_{max}}{K_m} \qquad [4.27]$$

4.6.1.2 Internal diffusion limitation

When biocatalysts are immobilised on the internal pore surfaces of a porous matrix, substrate diffuses through a tortuous pathway among pores and reacts with the enzyme immobilised on the pore surfaces. Diffusion and reaction occur simultaneously (see Fig. 4.20) and consequently a radial concentration gradient will develop in the particle in addition to the gradient in the thin stagnant film surrounding the particle. Therefore the reaction rate (assuming Michaelis–Menten kinetics and no partition effects) will be lower as well. This phenomenon is accounted for by the internal effectiveness factor, η_i (–). The internal effectiveness factor can be described as the observed reaction rate divided by the rate which would be observed if all biocatalyst experienced a substrate concentration equal to that at the surface of the porous biocatalyst bead:

$$\eta_i = \frac{-r_s}{-r_s(C_{s,surf})} = \frac{4\pi R_p^2 D_{eff}\left(\frac{\partial C_s(r)}{\partial r}\right)_{r=R_p}}{-r_s(C_{s,surf})} \qquad [4.28]$$

$\partial C_s(r)/\partial r$ at the surface of the particle is determined by the kinetics of the enzyme. Assuming Michaelis–Menten kinetics, no partitioning effects, and that the enzyme is distributed homogeneously over the porous support, we can write in the case of a steady state:

$$D_{\mathrm{eff}}\left(\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial C_s}{\partial r}\right)\right)=\frac{V'_{\max}C_s}{K_m+C_s} \qquad [4.29]$$

where V'_{max} is V_{max} per m^3 immobilisation material. Equation [4.29] can be solved numerically using the appropriate boundary conditions ($C_s = C_{s,surf}$ at $r = R_p$; and $\partial C_s(r)/\partial r = 0$ at $r = 0$), in order to determine $\partial C_s(r)/\partial r$ at the surface.

In order to quantify the internal effectiveness factor, the Thiele modulus (Φ) is often used (Aris, 1965; Bischoff, 1965). The Thiele modulus is the ratio of the kinetic rate and the diffusion rate:

$$\Phi=\frac{R_p}{3}\frac{-r_s(C_{s,\mathrm{surf}})}{\sqrt{2}}\left[\int_{C_s^*}^{C_{s,\mathrm{surf}}}D_{\mathrm{eff}}(-r_s)\,\mathrm{d}C_s\right]^{-0.5} \qquad [4.30]$$

C^* is the concentration when thermodynamic equilibrium is reached. In case of Michaelis–Menten kinetics, C^* equals zero. For Michaelis–Menten kinetics the Thiele modulus is defined as:

$$\Phi=\frac{R_p}{3}\sqrt{\frac{\dfrac{V'_{\max}C_{s,\mathrm{surf}}}{K_m+C_{s,\mathrm{surf}}}}{2D_{\mathrm{eff}}(K_m+C_{s,\mathrm{surf}})\left(1+\dfrac{K_m}{C_{s,\mathrm{surf}}}\ln\left(\dfrac{K_m}{K_m+C_{s,\mathrm{surf}}}\right)\right)}} \qquad [4.31]$$

For zero-order and first-order kinetics ($C_s >> K_m$ and $C_s << K_m$, respectively) analytical solutions do exist (for details see Van 't Riet and Tramper, 1991). A plot of η_i versus the Thiele modulus results in a generalised figure (see e.g. Aris, 1965; Bischoff, 1965; Van't Riet and Tramper, 1991). In scientific literature simplified expressions of the Thiele modulus are used as well (Shuler and Kargi, 2002); however these expressions do not lead to a generalised relationship between the Thiele modulus and the internal effectiveness factor.

Internal diffusion limitation does not only affect the transport of substrate into the immobilised biocatalyst particle, but also the transport of the product(s) formed by the biocatalyst to the bulk liquid. As a consequence of the formation of products in the particle, there will be a net flow of product(s) from the centre of the particle towards the surface of the immobilised biocatalyst particle. A radial concentration gradient will develop in the particle and additionally a concentration gradient will develop in the stagnant liquid film surrounding the particle. The average product concentration in the microenvironment of the biocatalyst is thus higher than the

concentration of product in the perfectly mixed bulk. If the kinetics of the system do not obey Michaelis–Menten kinetics but do obey product inhibition kinetics, internal diffusion limitation of the substrate and the product need to be taken into account in order to design and optimise the immobilised biocatalyst particle.

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4.7 References and further reading

Agrawal P.B., Nierstrasz V.A. and Warmoeskerken M.M.C.G. (2002) 'Bioscouring of cotton textiles: the structure of cotton in relation to enzymatic scouring processes,' *Proceedings of the 2nd Autex Conference*, Bruges, Belgium, 1–3 July, p. 562.

Apfel R.E. (1981) *Methods in Experimental Physics*, ed Edmonds P.D. Academic Press, New York, Vol.19 pp. 355–413.

Aris R. (1965) 'A normalisation for the Thiele modulus,' *Ind. Eng. Chem. Fundamental*, **4**, 227–229.

Bayer Textile Processing Chemicals (Product Guide).

Bird R.B., Stewart W.E. and Lightfoot E.N. (1960) *Transport Phenomena*, Wiley, New York.

Bischoff K.B. (1965) 'Effectiveness factors for general rate equation forms,' *AIChE J.*, **11** (2), 351–355.

Buchert J., Pere J., Puolakka A. and Nousiainen P. (2000) 'Scouring cotton with pectinases, proteases and lipases', *Textile Chem. Col. Am. Dyestuff Rep.*, **32** (5), 48–52.

Buschle-Diller G., El Mogahzy Y., Inglesby M.K. and Zeronian S.H. (1998) 'Effects of scouring with enzymes, organic solvents, and caustic soda on the properties of hydrogen peroxide bleached cotton yarn,' *Textile Res. J.*, **68** (12), 920–929.

Carslaw H.S. and Jaeger J.C. (1989) *Conduction of Heat in Solids*, Oxford University Press, Oxford.

Cavaco-Paulo A. (1998), *Processing textile fibres with enzymes: An overview*, ACS Symposium Series, **687**, pp. 180–189.

Cavaco-Paulo A., Almeida L. and Bishop D. (1996) 'Effects of agitation and endoglucanase pretreatment on the hydrolysis of cotton fabrics by a total cellulase,' *Textile Res. J.*, **66** (5), 287–294.

Cavaco-Paulo A., Cortez J. and Almeida L. (1997) 'The effect of cellulase treatment in textile washing processes,' *J. Soc. Dyers Soc.*, **117**, 17–21.

Cavaco-Paulo A., Almeida L. and Bishop D. (1998) 'Hydrolysis of cotton cellulose by engineered cellulases from *Trichoderma reesei*,' *Textile Res. J.*, **68** (4), 273–280.

Contreras R.R.D. (2001) 'Biopreparación: la nueva y avanzada forma de preparar hilo y tela (primera parte)', *Rev. Latinoamericana de Tech. Textile*, **14**, 55–60.

Cortez J.M., Ellis J. and Bishop D.P. (2001) 'Cellulase finishing of woven, cotton fabrics in jet and winch machines,' *J. Biotechnol.*, **89** (2–3), 239–245.

Cortez J.M., Ellis J. and Bishop D.P. (2002) 'Using cellulases to improve the dimensional stability of cellulose fabrics', *Textile Res. J.*, **72** (8), 673–680.

Crank J. (1956) *The Mathematics of Diffusion*, Clarendon Press, Oxford.

Csiszár E., Losonczi A., Szakács G., Rusznák I., Bezúr L. and Reicher J. (2001) 'Enzymes and chelating agents in cotton pre-treatment', *J. Biotechnol.*, **89** (2–3), 271–279.

Daniels F. and Alberty R.A. (1975) *Physical Chemistry*, 4th Edition, Wiley & Sons, New York.

Etters J.N. (1980) 'Diffusion equations made easy', *Textile Chem. Color.*, **12** (6), 140–145.

Etters J.N. (1999) 'Cotton preparation with alkaline pectinase: an environmental advance,' *Textile Chem. Color. Am. Dyestuff Rep.*, **1** (3), 33–36.

Farber P. and Dahmen N. (1998) 'About fluid flow and mass transfer in textile finishing machines', *Proceedings of FEDSM'98*, 1988 ASME Fluid Engineering Division Summer Meeting, Washington DC, June 21–25, 1–7.

Farber P., Dahmen N. and Mohaupt H. (1999) 'Virtual prototyping in textile finishing: academic pastime or industrial reality?' *Melliand Internat.*, **5** (1), 75–78.

Genencor International (Product Guide).

Gooijer H. (1998) *Flow resistance of textile materials*, PhD Thesis, University of Twente, The Netherlands.

Hartzell M.M. and Hsieh Y.L. (1998) 'Enzymatic scouring to improve cotton fabric wettability', *Textile Res. J.*, **68** (4), 233–241.

Heine E. and Höcker H. (1995) 'Enzyme treatments for wool and cotton', *Rev. Prog. Coloration*, **25**, 57–63.

Lange N.K. (2000) 'Biopreparation in action,' *Int. Dyer*, **185** (2), 18–21.

Lange N.K. and Henderson L. (2000) 'Biopreparation of cotton,' *1st Symposium on Textile Biotechnology in Textile Industry*, Povoa de Varzim, Portugal, 3–7 May.

Lenting H.B.M. and Warmoeskerken M.M.C.G. (2001a) 'Mechanism of interaction between cellulase action and applied shear force', a hypothesis, *J. Biotechnol.*, **89** (2–3), 217–226.

Lenting H.B.M. and Warmoeskerken M.M.C.G. (2001b) 'Guidelines to come to minimised tensile strength loss upon cellulase application', *J. Biotechnol.*, **89** (2–3), 227–232.

Lenting H.B.M., Zwier E. and Nierstrasz V.A. (2002) 'Identifying important parameters for a continuous bioscouring process', *Textile Res. J.*, **72** (9), 825–831.

Li Y. and Hardin I.R. (1998) 'Enzymatic scouring of cotton-surfactants, agitation and selection of enzymes', *Textile Chem. Color.*, **30** (9), 23–29.

Mason T.J. (1990) *Sonochemistry the Uses of Ultrasound in Chemistry*, Royal Society of Chemistry, Cambridge.

Mason T.J. and Lorimer J.P. (1989) *Sonochemistry, Theory, Applications and Uses of Ultrasound in Chemistry*, Ellis Horwood, Chichester.

McCall R.E., Lee E.R., Mock G.N. and Grady P.L. (1998) 'Improving dye yields of vats on cotton fabric using ultrasound', *AATCC International Conference and Exhibition*, Book of abstracts, pp. 188–194.

Mielgo I., Moreira M.T., Feijoo G. and Lema J.M. (2001) 'A packed-bed fungal bioreactor for the continuous decolourisation of azo-dyes (Orange II)', *J. Biotechnol.*, **89** (2–3), 99–106.

Mielgo I., Moreira M.T., Feijoo G. and Lema J.M. (2002) Biodegradation of a polymeric dye in a pulsed bed bioreactor by immobilised *Phanerochaete chrysosporium*, *Water Res.*, **36** (7), 1896–1901.

Moholkar V.S. (2002) *Intensification of textile treatments: sonoprocess engineering*, PhD Thesis, University of Twente, The Netherlands.

Moholkar V.S. and Pandit A.B. (2001) 'Modeling of hydrodynamic cavitation reactors: a unified approach,' *Chem. Eng. Sci.*, **56** (21–22), 6295–6302.

Moholkar V.S. and Warmoeskerken M.M.C.G. (2000) 'Mechanistic studies in ultrasonic textile washing', *AATCC Annual Book of Papers*, Section 18, pp. 1–8.

Moholkar V.S. and Warmoeskerken M.M.C.G. (2001) 'Intensification of mass transfer in textile materials', *Proceedings of the 1st Autex Conference (Technitex)*, Povoa do Varzim (Portugal), June 26–29, pp. 204–213.

Moholkar V.S. and Warmoeskerken M.M.C.G. (2002) 'Mechanistic aspects and optimization of ultrasonic washing', *AATCC Rev.*, **2** (2), 34–37.

Moholkar V.S., Rekveld S. and Warmoeskerken M.M.C.G. (2000) Modeling of the acoustic pressure fields and the distribution of the cavitation phenomena in a dual frequency sonic processor, *Ultrasonics*, **38**, 666–670.

Moholkar V.S., Huitema M., Rekveld S. and Warmoeskerken M.M.C.G. (2002) Characterization of an ultrasonic system using wavelet transforms, *Chem. Eng. Sci.*, **57** (4), 617–629.

Neppiras E.A. (1980) 'Acoustic cavitation', *Phys. Rep.*, **61**, 159–251.

Novozymes (Product Information sheet).

Oxspring D.A., McMullan G., Smyth W.F. and Marchant R. (1996) 'Decolourisation and metabolism of the reactive dye', Remazol Black B, by an immobilised microbial consortium, *Biotechnol. Lett.*, **18** (5), 527–530.

Pere J., Puolakka A., Nousiainen P. and Buchert. J. (2001) 'Action of purified *Trichoderma reesei* cellulases on cotton fibers and yarn,' *J. Biotechnol.*, **89** (2–3), 247–255.

Rathi H., Mock G.N., McCall R.E. and Grady P.L. (1997) 'Ultrasound aided open width washing of mercerised 100% cotton twill fabric,' *AATCC International Conference and Exhibition*, Book of abstracts, pp. 254–262.

Rehm H.J., Pühler A. and Stadtler P. (1996), *Biotechnology. A Multi-Volume Comprehensive Treatise. Vol. 6: Products of Primary Metabolism*, Weinheim: VCH-Verlagsgesellschaft.

Shuler M.L. and Kargi F. (2002) *Bioprocess Engineering: Basic Concepts*. 2nd Edition, Prentice Hall PTR, New York.

Stanescu M.D. (2002), 'Enzymes in textile finishing,' *Proceedings of the 2nd Autex Conference*, Bruges, Belgium, 1–3 July, 446–453.

Sun S.F. (1994) *Physical Chemistry of Macromolecules: Basic Principles and Issues*, Wiley & Sons, New York.

Suslick K.S. (1988) *Ultrasound: Its chemical, physical and biological effects*, VCH, New York.

Thakore K.A. (1990) Physico-chemical study on applying ultrasonics in textile dyeing, *Am. Dyestuff Rep.*, **79** (5), 45–47.

Thiry M.C. (2001), 'Enzymes in the toolbox,' *AATCC Rev*, **1** (8), 14–19.

Tzanov T., Calafell M., Gübitz G.M. and Cavaco-Paulo A. (2001) 'Bio-preparation of cotton fabrics', *Enzyme Microbiol. Technol.*, **29**, 357–362.

Van den Brekel L.D.M. (1987) *Hydrodynamics and mass transfer in domestic drum type washing machines*, PhD Thesis, TU Delft, The Netherlands.

Van den Brekel L.D.M. and de Jong E.J. (1988) ‘Hydrodynamics in packed textile beds’, *Textile Res. J.*, **59**, 433.

Van der Donck J.C.J., So A. and Frens G. (1998) ‘The influence of stretching on salt release from porous yarns’, *Tenside Surfactants Detergents*, **35**, 119–122.

Van Holde K.E. (1971) *Physical Biochemistry*, Prentice Hall, New Jersey.

Van ’t Riet K. and Tramper J. (1991) *Basic Bioreactor Design*, MDI, New York.

Waddell R.B. (2002) ‘Bioscouring of cotton: commercial applications of alkaline stable pectinase’, *AATCC Rev.*, **2** (4), 28–30.

Warmoeskerken M.M.C.G., Haverkamp J., Simon W. and Van der Vlist P. (1994) *A process for cleaning articles whereby soiled articles are immersed in an enzymatic aqueous cleaning medium and radiated with ultrasonic energy, characterized in that the enzymatic aqueous cleaning medium contains an enzyme having lipolytic activity*, Patent, WO9407989.

Warmoeskerken M.M.C.G., Van der Vlist P., Moholkar V.S. and Nierstrasz V.A. (2002), ‘Laundry process intensification by ultrasound’, *Colloids and Surfaces A*: Physicochem. Eng. Aspects, **210** (2–3), 277–285.

Warmoeskerken M.M.C.G. and Boom R.M. (1999) *90th AOCS Annual Meeting & Expo*, May 9–12, Orlando, USA.

Yachmenev V.G., Blanchard E.J. and Lambert A.H. (1998) ‘Study of the influence of ultrasound on enzymatic treatment of cotton fabric’, *AATCC Book of Papers*, 472–481.

Yachmenev V.G., Blanchard E.J. and Lambert A.H. (1999) Study of the influence of ultrasound on enzymatic treatment of cotton fabric, *Textile Chem. Color. Am. Dyestuff Rep.*, **1** (1), 47–51.

Yachmenev V.G., Bertoniere N.R. and Blanchard J. (2001) ‘Effect of sonification on cotton preparation with alkaline pectinase’, *Textile Res. J.*, **71** (6), 527–533.