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Fungal Decolourization and Degradation of Synthetic Dyes Some Chemical Engineering Aspects

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1. Introduction

There are more than 100,000 different synthetic dyes available on the market, produced in over 700,000 tons annually worldwide. They are used in the textile, paper, cosmetics, food and pharmaceutical industries. Some of them are dangerous to living organisms due to their possible toxicity and carcinogenicity. About 10% of the above mentioned amount is lost in wastewater, which justifies the concern about the environment. Among the numerous water-treatment technologies, research interest in the fungal bioremediation, i.e. decolourization and degradation of synthetic dyes, has increased significantly in the last three decades.
The physico-chemical methods of dye degradation have already been well recognized from the chemical engineering point of view and also widely applied on the industrial scale. In the last few decades, research in the dye bioremediation technologies has gained its significance. From the available literature, it can be seen that the majority of research has been performed from the biochemical and microbiological point of view on a laboratory scale, while there is a lack of chemical engineering approach to the research of this serious problem. The purpose of this work is to review the chemical engineering principles, which should be applied during the research and transfer of dye bioremediation technologies to a large scale. Accordingly, a brief review of research results from bioreactors of volumes larger than 1.0 L is presented.

2. Alternative technologies

The dyes in wastewaters present a significant problem in the wastewater treatment, due to the complex and varied chemical structure of these compounds along with other residual chemical reagents and impurities. Generally, organic contents are high, while the BOD/COD ratios are low due to the not easily degradable nature of dyes. In addition, the degradation of products may be toxic. According to the latter, no universal method is known for their treatment. The degradation of synthetic dyes in waste streams can be performed with various technologies, which can be subdivided into four main groups: 1) physical, 2) chemical and photochemical, 3) electrochemical, and 4) biological processes. The processes are presented in Table 1 and briefly described below (Robinson et al, 2001; Joshi et al, 2004; Singh, 2006).

Regulatory agencies, esp. in developed countries, are concerned with environmental and public health, and with the imposition of the stringent environmental legislation, which is increasingly causing problems for the textile and dyestuff industry. The legislation and colour standards for waste discharge vary in different states. In addition, there are several standard methods for determining the colour standards, which aggravates a comparison of different colour degradation methods from various sources (Hao et al, 2000; Singh, 2006).

2.1 Physical methods

Adsorption has gained a favourable interest due to the efficient pollutant removal, quality product and economical feasibility. It is influenced by many physico-chemical factors, e.g. dye-sorbent interaction, adsorbent surface area and particle size, temperature, pH and contact time. Materials, like activated carbon, peat, wood chips, fly ash and coal, silica gel, microbial biomass, and other inexpensive materials (e.g. natural clay, corn cobs, rice hulls), are used, since they do not require regeneration. *Sedimentation* is a solid-liquid separation method. In the case of dye solutions, it is used in a combination with chemical or biological methods producing particles containing dye or dye degradation products with coagulation/precipitation or with some other chemical methods, or adsorption on various materials. The rate of sedimentation of particles suspended in a fluid can be described with Stoke's law and is influenced by many physico-chemical factors. The disadvantage here is a high sludge production. *Flotation* is a foam separation technique. Generally, it is performed by adding a surface active ion of the opposite charge to the ion to be separated from the solution. The solid product which appears on the gas-liquid surface is levitated to the surface of the solution by means of a gentle stream of fine gas bubbles. *Coagulation* can be induced by an electrolytic reaction at electrode surface or by changing pH or adding coagulants (Shakir et al, 2010). Furthermore, *membrane filtration* can be used to remove dye molecules. The classification of membranes is conducted on the basis of their pore size to retain solutes with different molecular weights. The membrane parameter is called molecular weight cut off (MWCO). In the case of dye separation, reverse osmosis (MWCO < 1000), nanofiltration (500 < MWCO < 15000) and ultrafiltration (1000 < MWCO < 100000) membranes can be used according to the dye characteristics. In addition to the dye solution separation, membranes can be used also for the separation of particles after the adsorption or coagulation/precipitation instead of the sedimentation (Hao et al, 2000). The *radiation* itself can be classified as a physical method. However, in the case of dye degradation, the radiation dose in aqueous media leads to the formation of strong oxidizing species such as $^{\bullet}OH$ radicals, which are able to react with dye molecules, degrade them and consequently,

Physical	**Chemical**	**Electrochemical**	**Biological**
Adsorption	H_2O_2 oxidation	Electrocoagulation	Bacterial aerobic
Sedimentation	Fenton oxidation	Electroflotation	Bacterial anaerobic
Flotation	Ozonization	Electrooxidation	Algae
Coagulation	Chlorination	Electroreduction	Fungi
Membrane filtration	Photochemical oxidation		Yeast
Radiation	Wet air oxidation		
	Reduction		

Table 1. Methods for dye degradation and decolourization in waste streams

enhance the degradation process. Therefore, radiation methods are usually included in the advanced oxidation processes (AOPs) (Rauf et al, 2009). During *ultrasonic irradiation*, the propagation of an ultrasound wave leads to the formation of cavitation bubbles. The collapse of these bubbles spawns high temperatures and pressures, which leads to the production of radical species and in consequence, to the chemical reaction of dye degradation (Vinodgopal et al, 1998). In general, solid waste disposal is required after the physical methods of separation.

2.2 Chemical and electrochemical methods

Chemical oxidation is the most commonly used method of decolourization, mainly due to its simplicity of application. The oxidising agent is usually hydrogen peroxide, which needs to be activated due to its stability in the pure form. Methods vary according to the way in which H_2O_2 is activated. It removes the dye from the effluent with an aromatic ring cleavage of dye molecules. A well known activator is Fe(II) salt known as Fenton's reagent. The result of sorption or bonding of dissolved dyes is a sludge generation through the flocculation of reagent and dye molecules, which needs disposal and is therefore disadvantageous. H_2O_2 can be activated also with ozonization. A major drawback is a short half-life of ozone in water and its cost – it degrades in about 20 minutes and has to be applied continuously. In addition, its stability is affected by the presence of dyes, salts, pH and temperature. Hydrogen peroxide can be activated also with UV radiation. The major advantage of H_2O_2/UV treatment is that the use of no other chemicals is required. The wet air oxidation (WAO) process presents a hydrothermal treatment of dissolved and suspended components in water, and has been successfully used also for several azo dyes (Kusvuran et al, 2004; Rodriguez et al, 2009). Chlorination, using chlorine gas or sodium hypochloride, is an inexpensive and effective method. It has become less frequent due to the generation of toxic and carcinogenic compounds. In addition, the use of chemicals containing chlorine is restricted due to environmental reasons. As already mentioned, photochemical methods are based on the use of UV light, which activates the chemicals and consequently, enhances the chemical reaction and makes the process more efficient.

The principle of electrochemical methods is to charge the electric current through electrodes made of different materials (e.g. iron or aluminium) resulting in the oxidation process at anode and reduction at the cathode with H_2 production. The resulting processes are known as electrocoagulation, electroflotation, electrooxidation and electroreduction. The majority of the above mentioned methods are the so-called 'advanced oxidation processes' (AOP), and are essentially based on the generation of highly reactive radial species (Hao et al, 2000; Slokar & Majcen, 1998; Joshi et al, 2004).

2.3 Biological methods

A biological treatment presents a degradation of organic substances by microorganisms under aerobic or anaerobic conditions, and has been widely used and researched. The dyes themselves are generally resistant to oxidative biodegradation. In addition, toxicity, as well as the acclimating ability is a drawback of using microbial cultures. It has been demonstrated that mixed bacterial cultures are capable of decolourizing textile dye solutions. Nevertheless, several studies show that little biodegradation actually occurs and that the primary mechanism is adsorption to the microbial biomass (Slokar, 1998; Robinson et al, 2001; Knapp, 2001).

A continuous aerobic or anaerobic treatment can be conducted in a variety of bacterial bioreactors, e.g. reactors with activated sludge, reactors with biofilm in the form of fixed bed, rotating discs or rotating drum. An aerobic and anaerobic treatment can also be combined. It has also been reported that few species of algae are capable of degrading azo dyes and utilize them as a sole source of carbon. Some articles on yeasts capable of dye decolourization can also be found in the literature (Joshi et al, 2004). Several fungal systems have been demonstrated to degrade various classes of dyes. A particular interest was devoted to the white-rot fungi and azo dyes, the largest class of commercial dyes. A fungal treatment of dyes is an economical and feasible alternative to the present treatment technologies (Knapp, 2001; Singh, 2006).

3. Dyes

The main common property of dyes is to absorb light due to the chromophore, a part of the molecule responsible for its colour. The colour arises when a molecule absorbs certain wavelengths of visible light and transmits or reflects the others. However, the variation in the structure is enormous and many thousand different dyes are produced for commercial use. In general, dyes can be classified according to their chemical structure, particularly chromophore, and the method of application. The classes of dyes from the textile industry together with some of their typical representatives are presented in Table 2 (Corbmann, 1983; Hao et al, 2000).

Classification according to chemical structure and/or chromophore	**Classification according to method of application**
azo	acid
anthraquinone	basic
triphenylmethane	direct
phthalocyanine	reactive
indigo	disperse
sulphur	vat
	mordant
	sulphur

Table 2. Main groups of dyes according to chemical structure and method of application

Among 12 different chromophores, azo and anthraquinone dyes are the major units. *Azo dyes*, characterized by nitrogen to nitrogen double bonds account for up to 70% of all textile dyestuff produced and are the most common chromophore of reactive dyes. *Anthraquinone dyes* derive from anthraquinone with a quinoid ring acting as the chromophore and either hydroxyl groups or amino groups attached to the general structure. *Triphenylmethane dyes* are synthetic organic dyes with a molecular structure based on the hydrocarbon triphenylmethane, used in textile applications where lightfastness is not important. The *phthalocyanine dyes* derive from the macrocyclic compound which forms a coordination complex with most elements of the periodic table. They are few in number, but commonly used. *Indigo* is an organic dye with a distinctive blue colour. Historically, it was extracted

from plants; however, nearly all indigo produced today is synthetic. *Sulphur* dyes are a group of sulphur-containing complex synthetic organic dyes (Hao et al, 2000).
Acid dyes are water soluble anionic dyes with different chromophore groups substituted with acidic functional groups such as nitro-, carboxyl- and sulphonic acid, for the dye to become soluble. *Basic dyes* are cationic types with chromophores typically having amino groups. *Direct dyes* are highly water-soluble salts of sulphonic acid of azo dyes. *Reactive dyes* are highly water-soluble anionic dyes with wet fastness and binding to textile fibres via covalent bonds. *Disperse dyes* are substantially water-insoluble non-ionic dyes for the application to the hydrophobic fibres from aqueous dispersions. *Sulphur dyes* are dyes applied in two parts. The initial bath consists of the yellow or pale chartreuse colour, which is aftertreated with a sulphur compound in place to produce dark black. *Mordant dyes* require a mordant (usu. potassium dichromate), which improves dyestuff fastness on a dyeing material in water media. Many mordants can be hazardous to health. *Vat dyes* are essentially insoluble in water and incapable of direct dyeing of fibres. A reduction in alkaline liquor makes them water soluble and attachable to textile fibres, while a subsequent oxidation reforms the originally insoluble dye (Corbmann, 1983).

4. Fungal decolourization and degradation of dyes

4.1 White-rot fungi

White-rot basidiomycetes are a group of fungi capable of depolymerizing and mineralizing otherwise not easily degradable lignin with their extracellular and non-specific ligninolytic enzymes. In the 1980s, this fact stimulated research on the ability of ligninolytic fungi to degrade organic pollutants (Pointing, 2001; Gao et al., 2010). It was established that *Phanerochaete chrysosporium* is capable of biodegrading various pollutants and it soon became a model white-rot fungus with most of the research done up to now. The enzymes produced with this fungus are lignin peroxidase (LiP) and manganese peroxidise (MnP)(Podgornik et al, 2001; Faraco et al, 2009). In the next decade, a few new species of white-rot fungi like *Pleurotus ostreatus* and *Trametes versicolour* (Heinfling et al, 1997; Sukumar et al, 2009; Pazarlioglu et al, 2010) were characterized for the dye degradation. A more intense research with *Irpex lacteus* (Novotny et al, 2009) and *Bjerkandera adusta* (Robinson et al, 2001; Eichlerova et al, 2007) started in the last decade, while the interest in the decolourization capability of *Ceriporiopsis subvermispora* (Babič & Pavko, 2007; Tanaka et al, 2009) and *Dichomites squalens* (Eichlerova et al, 2006; Pavko & Novotny, 2008) has increased in the last few years.

Organism	Enzyme activities	Reference
Bjerkandera adusta	MnP, Lac, LiP	Robinson et al, 2001; Eichlerova et al, 2007
Ceriporiopsis subvermispora	MnP,Lac	Babič & Pavko, 2007; Tanaka et al, 2009
Dichomitus squalens	MnP, Lac	Eichlerova et al, 2006; Pavko & Novotny, 2008
Irpex lacteus	MnP, Lac, LiP	Novotny, 2009
Phanerochaete chrysosporium	LiP, MnP	Podgornik et al, 2001; Faraco et al, 2009
Pleurotus ostreatus	LiP, Lac	Heinfling et al,1997; Sukumar et al, 2009; Pazarlioglu et al, 2010
Trametes versicolour	Lac, LiP	Heinfling et al,1997; Sukumar et al, 2009; Pazarlioglu et al, 2010

Table 3. Some white-rot fungi used in biodegradation/decolourization studies and their most commonly expressed enzyme activities

Some white-rot fungi used in the biodegradation/decolourization studies and their most commonly expressed enzyme activities are presented in Table 3. The data are collected from numerous research articles, where the cultivation conditions varied and it is thus possible that an activity would or would not occur under different cultivation conditions, esp. nitrogen contents (Knapp, 2001; Singh, 2006).

4.2 Mechanisms of fungal dye degradation and decolourization

The mechanisms of fungal dye decolourization and degradation are listed in Table 4. The accumulation of chemicals with the microbial biomass is termed *biosorption*, and can take place on living or dead biomass. Waste and/or dead microbial biomass can be used as an efficient adsorbent, especially if containing a natural polysaccharide chitin and its derivative chitosan in the cell walls. Chitosan, a cell wall component of many industrially useful fungi, has a unique molecular structure with a high affinity for many classes of dyes (Joshi et al, 2004).

Adsorption (biosorption)
Biodegradation
Adsorption and biodegradation
Mineralization
Utilization as carbon source

Table 4. Mechanisms of fungal dye degradation and decolourization

It is known that most of the white-rot fungi produce at least two of the three highly nonspecific enzymes like LiP, MnP and Lac, which enable the generation of free radicals when conducting a variety of reactions (Pointing, 2001; Knapp, 2001). The structure of dyes strongly influences their degradability by pure cultures and isolated enzymes. Numerous data about biodegradation of various synthetic dyes with selected white-rot fungi have been published. Nevertheless, a limited number of data are available on systematic studies about the relation between the structure and biodegradability, esp. for commercial dyes with a complex structure. According to the above mentioned, in the presence of biomass in the dye solution, it has to be distinguished between the dye depletion due to adsorption and the one due to enzymatic degradation. The fungal action rarely leads to the mineralization of dyes and very much depends on the chemical structure. A higher mineralization occurs with dyes containing substituted aromatic rings in their structure compared to the unsubstituted rings. A better mineralization is observed also under nitrogen limited conditions. Some reports on the utilization of dyes as a carbon source have been published in the last decade. Certain bonds in the dye molecule are cleaved and utilized as a carbon source, the chromophore not being affected. This mechanism occurs preferably in the consortium of microorganisms (Knapp, 2001; Singh, 2006).

4.3 Factors affecting fungal decolourization and degradation of dyes

The fungal growth and enzyme production, and consequently, decolourization and degradation are influenced by numerous factors, e.g. media composition, pH value, agitation and aeration, temperature and initial dye concentration. Their effect is briefly presented and discussed below.

4.3.1 Media composition

There is no doubt that media composition has an enormous effect on fungal growth and production of their decolourization systems. It must be noted that real industrial effluents vary with location and time, not to mention the often very complex composition with a lack of nutrients, compared to the usually well defined media used in the research. Therefore, attention has to be focused on the supply of carbon and nitrogen sources together with mineral nutrients and other additives (Hao et al, 2000; Knapp, 2001; Singh, 2006).

Carbon source. A carbon source is necessary for fungal growth and to provide the supply for oxidants, the fungus requires for decolourization. Glucose has been used in the majority of research studies. Alternatives are fructose, sucrose, maltose, xylose and glycerol, while also starch and xylan seem to be useful. Surprisingly, cellulose and its derivatives were not effective. For initial experiments, glucose at 5–10 g/L is a good choice. Effluents from dyeing or chemical/dye production usually do not contain usable carbon substrates, while others from distilling or paper pulping may have a range of carbohydrates as useful substrates for certain white-rot fungi. The need to add carbon source depends on the organism and type of the dye to be treated.

Nitrogen source. The nitrogen demand for growth and especially enzyme production differ markedly among fungal species. It is well known that the production of ligninolytic enzymes with *P. chrysosporium* is much more effective under the conditions of nitrogen limitation. On the other hand, *B. adusta* produces more LiP and MnP in nitrogen-sufficient media. White-rot fungi can use inorganic as well as organic nitrogen sources. Inorganic nitrogen, in most cases ammonium salts, has been used during the research of fungal growth and enzyme production, since the organic nitrogen seems not to be advantageous. In the case of effluents, the presence of usable nitrogen sources should be considered.

Other media components. Many studies have been using growth factors. However, considering their expense, it is not economical to use them in the decolourization technologies. All microbes have certain requirements for mineral nutrients, e.g. white-rot fungi need iron, copper and manganese. They can be a part of the effluent or must be added to the media. A variety of other materials, like veratryl alcohol, tryptophan and aromatics, e.g. phenol and aniline, can act as low molecular mass redox mediators of ligninolytic activities and therefore promote the decolourization (Knapp, 2001; Singh, 2006). It is interesting that some components in wood and straw induce the enzyme production with white-rot fungi. For example, the enzyme activity ratio Lac/MnP can be regulated using beech wood as the immobilization support and inducer together with a combination of various concentrations of additional nitrogen and carbon source in the liquid media during the cultivation of *Ceriporiopsis subvermispora* (Babič & Pavko, 2007). The ligninolytic enzyme production by *Dichomitus squalens* can be substantially induced by adding beech wood and straw particles to the liquid media (Pavko & Novotny, 2008).

4.3.2 pH

Most of the research on growth and enzyme production has been performed in batch cultures, usually without the pH control during the cultivation, for the influence of initial pH value, sometimes with adequate buffering, to be investigated. The majority of filamentous fungi together with white-rots grow optimally at acidic pH values. Depending on the used substrate, pH changes during cultivation. The growth on carbohydrate-containing media generally causes acidification of the media, which depends on the carbon source and present buffering. The decolourization can be conducted with a whole

fermentation broth (mycelium and enzymes) or with isolated enzymes. It has to be distinguished between the optimum pH for growth and enzyme production, the optimum pH for the action of isolated enzymes and the optimum pH for dye degradation. Therefore, optimum pH depends on the medium, fungus and its enzyme system, as well as on the decolourization under consideration. The majority of researchers suggest that the optimum pH values are likely to be in the range 4–4.5 (Knapp, 2001).

4.3.3 Temperature

Temperature has to be considered from various viewpoints: its influence on the growth and enzyme production, the enzymatic decolourization rate and the temperature of the waste stream. Most white-rot fungi are mesophiles with the optimal cultivation temperature 27–30 °C. The optimal temperatures for enzyme reactions are usually higher, but the enzyme instability and degradation has to be taken into account at temperatures approaching for example 65 °C. Various textile and dye effluents are produced at temperatures 50–60 °C. The optimal decolourization process temperature for a particular process has to be thus selected from case to case according to the mentioned parameters (Knapp, 2001; Singh, 2006).

4.3.4 Agitation and aeration

Ligninolytic fungi are obligate aerobes and therefore need oxygen for growth and maintenance of their viability. In addition, lignin degradation also requires oxygen, either for the mycelial generation of H_2O_2 for peroxidases or for the direct action of oxidases. Oxygen could also act directly on lignin fragments. The oxygen demand depends on the fungus and its ligninolytic system.

The oxygen supply to the culture media during the cultivation has been an interesting research topic for decades and has been covered in numerous articles. The major problem is its low water solubility, which is only 8 mg/L at 20 °C. To satisfy the microbial oxygen requirements during the cultivation and to enhance the oxygen gas-liquid mass transfer, the aeration and agitation are necessary. This might affect the morphology of filamentous fungi and lead to the decreased rate of enzyme synthesis (Žnidaršič & Pavko, 2001). As a result, various bioreactor types generally divided into static and agitated configurations were invented to provide enough oxygen. The choice of the reactor depends on the particular system although an appropriate agitation gives as good or even better results as those from static conditions. Particular studies on the effect of agitation and aeration only on the decolourization process were not found in the literature (Knapp, 2001).

4.3.5 Initial dye concentration

It is important to optimize the initial dye concentration for colour removal. Dyes are namely usually toxic to microorganisms, while the toxicity depends on the type of dye. Higher dye concentrations are always toxic. The range of initial dye concentrations studied in the literature generally varies from 50–1000 mg/L, and depends on the investigated microorganism and type of dye (Singh, 2006).

5. Reactor design considerations

The reactor design uses information, knowledge and experience from various areas, e.g. thermodynamics, chemical kinetics, fluid mechanics, mass and heat transfer, and economics.

To select a proper reactor configuration and size, it is necessary to know how materials flow and contact in the reactor, and how fast is the process. Usually, the conversion of the reactant should be as high and as fast as possible at the lowest costs (Levenspiel, 1999).
In our case, two different processes are taking place. The first one is the fungal growth and enzyme synthesis with fungal biomass, and the second one is the decolourization and degradation of dyes caused by the produced enzymes. Fungal enzymes can be intracellular or extracellular products, synthesized during the growth or after the growth phase. The colour depletion in wastewater can take place due to the enzymatic degradation or only adsorption on the biomass. The enzyme production and decolourization must be simultaneously optimized to get the best dye conversion in the shortest time. According to this, two main strategies can be pursued, i.e. (1) the direct transformation of dye with the active biomass in one reactor, or (2) the use of extracted enzymes from the culture medium.
The biomass growth and enzyme production as well as dye degradation take place in the liquid phase. Under aerobic conditions, the aeration of the reactor is necessary, while under anaerobic conditions, methane is produced. The microbial biomass, especially when immobilized, can be treated as a solid phase; therefore, the reactor can be considered as a gas-liquid-solid system with all its liquid flow and mass transfer characteristics.
For a successful design of the process with a given capacity, the reactor type, i.e. shape and size, as well as the operation mode of the reactor must be selected at the beginning. Moreover, the operating conditions such as concentrations, flow rates, temperature and pH must be defined. One of the most important data for the design is the reaction rate, which allows the calculation of time in the batch mode or flow throughput in a continuous mode for the necessary reactant conversion – in our case, dye degradation or decolourization degree. Frequently, pilot plant experiments in addition to laboratory data are necessary to establish the proper scale up method for the transfer of the process from the laboratory to industrial scale. Finally, an economical evaluation is crucial before the realization of the project. All these facts are briefly described in the continuation of this chapter.

5.1 Reaction rate

In the chemical reactor design, chemical reactions can be usefully classified into homogeneous and heterogeneous reactions according to the present gas, liquid and solid phases. In addition, a distinction can be made between non-catalytic and catalytic reactions. The reaction rate of the reaction component is usually based on the unit volume of reaction fluid (mol/Ls); however, it can be based on the unit mass of catalyst, unit interfacial surface in the heterogeneous system etc. On the other hand, a chemical reaction follows the stoichiometric equation. In the simplest case of a homogenous reaction of components A and B in a liquid phase:

$$aA + bB \rightarrow \text{products} \tag{1}$$

the expression for the reaction rate of disappearance of the component A can be written as follows:

$$dC_A/dt = -r_A = k\, C_A^a C_B^b = k\, C_A^a (b/a)^b C_A^b = kC_A^{a+b} = kC_A^n \tag{2}$$

where $k = k(b/a)^b$ is the reaction rate constant, a and b are the reaction order with respect to A and B – the power to which the concentration is raised, while n is the overall reaction order. The integrated form of the equation allows the calculation (prediction) of time

required to achieve the desired degradation level or conversion for the component A (e.g. dye) in a batch reactor for a given concentration at the beginning (C_{A0}) and the end (C_A) of the process:

$$t = -\int_{C_{A0}}^{C_A} dC_A / (-r_A) \tag{3}$$

For elementary reactions, the correlation between the stoichiometric and rate equation is simple, while with non-elementary reactions, the reaction mechanism must be known to write down the correct rate equation. The enzyme-substrate reactions are a typical case (Levenspiel, 1999). With heterogeneous reactions, the oxygen transfer from air bubbles to the liquid phase or the transport of dye from the bulk solution inside the biomass particle and its degradation has to be considered. In this case, the rate expression and the design equation to predict the conversion in the reactor of selected size and operating conditions are much more complicated.

Figure 1 shows that the shape of the concentration vs. time curve varies with the reaction order (n = 0 : k = 1.2 mg/Lh; n = 1 : k = 0.1 h^{-1}; 0 < n < 1 : k_1 = 1 h^{-1}, k_2 = 1 mg/L). A mathematical analysis of the experimental data allows the determination of the kinetic Equation 2.

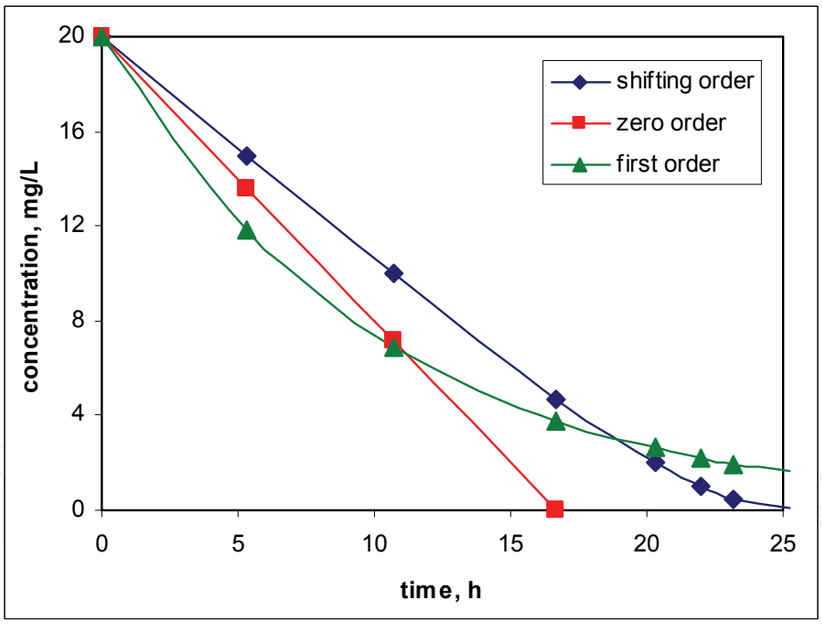

Fig. 1. Concentration profile of reactant following reaction of zero and first order, and for shifting order (enzyme catalysed reaction following Monod kinetics) in batch reactor

5.2 Fluid flow

Ideal reactors have three ideal flow or contacting patterns. In a well mixed batch reactor, a uniform composition is everywhere in the reactor, but the composition and consequently, the reaction rate changes with time. For a continuous operation, two types of ideal flow can be achieved. In a mixed flow, the same composition is everywhere within the reactor and

also at the exit. Furthermore, the reaction rate is the same at any point in the reactor. Therefore, the performance equation can be written for the reactor as a whole:

$$\tau = V/F = (C_{A0} - C_A)/(-r_A) \quad (4)$$

where τ (s) is the space time or time required to process one reactor volume of feed measured at specified conditions, V (m^3) is the fluid volume in the reactor, F is the volumetric flow (m^3/s), C_{A0} and C_A are the concentrations of reactant in the inlet and outlet stream, while $-r_A$ (g/m^3s) is the expression for the reaction rate of disappearance of the reactant – in our case dye.

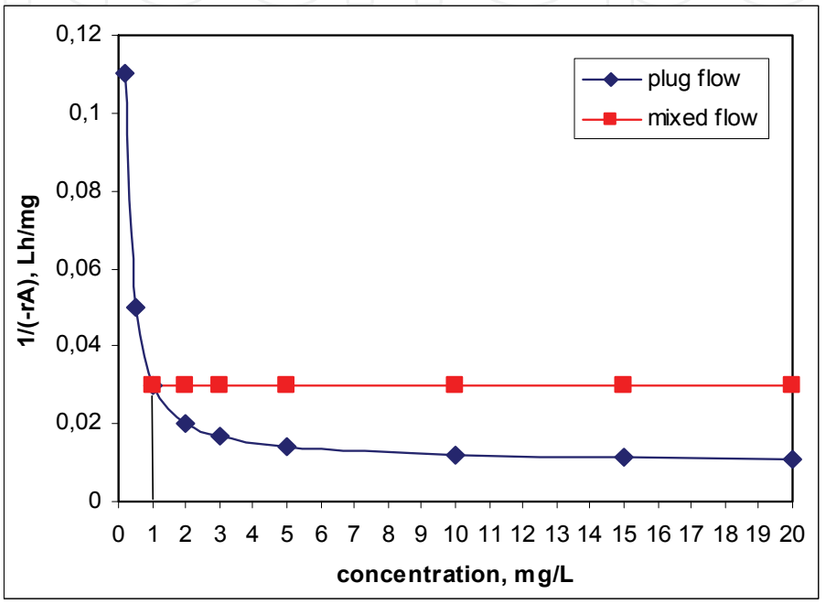

Fig. 2. Effect of ideal fluid flow on reactor performance: for same inlet and outlet concentrations, and fluid flow rate, volume of plug flow reactor is smaller

In the reactor with a plug flow, the composition of the fluid varies from point to point along the flow path. To obtain the performance equation for the whole reactor, an integration of differential mass balance equation for a differential volume element is necessary.

$$\tau = V/F = -\int_{C_{A0}}^{C_A} dC_A/(-r_A) \quad (5)$$

In the above equation, τ (s) is the space time or time required to process one reactor volume of feed measured at specified conditions, V (m^3) is the fluid volume in the reactor, F is the volumetric flow (m^3/s), C_{A0} and C_A are the concentrations of reactant in the inlet and outlet stream, while $-r_A$ (g/m^3s) is the expression for the reaction rate of disappearance of the reactant, in our case dye.

Typical shapes of reactors where the plug flow can be achieved are tubular reactors or long columns. A consequence of the type of flow is that the reactor volume for mixed flow and plug flow (for reaction order not equal zero) is different for the same fluid throughput and reactant inlet concentrations and its conversion at the exit. This very important fact has to be

considered in the reactor selection and design. Figure 2 shows the case for the Michaelis-Menten enzyme kinetics (v_{max} = 100 mg/Lh, K_m = 2.0 mg/L, C_{A0} = 20 mg/L, C_A = 1 mg/L). It can be seen that the space time and consequently, the volume for the mixed flow reactor is proportional to the area below the horizontal line (cf. Equation 4), while for the plug flow reactor, the space time is proportional to the area below the curve (cf. Equation 5). In consequence, the required volume of plug flow reactor is by 50% lower (Doran, 1995; Levenspiel, 1999).

Real reactors always deviate from an ideal flow due to stagnant regions, fluid channelling, or short-circuiting. To predict the behaviour of a continuous reactor, the velocity distribution of fluid elements or their residence time distribution (RTD) has to be considered. This information can be obtained easily and directly with stimulus-response experiments. In a two phase reactor, stirred tank with aeration or bubble column for example, each phase can have its own flow characteristics (Levenspiel, 1999).

5.3 Types of operation

In general, two basic modes of reactor operation are known. A constant volume batch operation is a typical non-steady state process. Here, the final concentration of the produced biomass and enzyme and/or degraded dye and degradation products in the reactor are achieved after a certain process time. During the constant volume continuous process, the biomass growth and enzyme production as well as dye degradation take place in the reactor with the reactant inflow, in our case substrate and/or dye, and liquid outflow with the products of the process. The outflow may contain biomass, produced enzyme, non-used substrate, non-degraded dye and dye degradation products. The type of flow must be considered and a steady state can be achieved. To increase the process performance, biomass and/or enzymes can be immobilized and kept in the reactor. Extracellular enzymes can be retained in the reactor by using a membrane, which permeates the dye with its degradation products. The concentrations at the liquid outflow mainly depend on the kinetics of growth, enzyme production and dye degradation as well as space time, defined as the reactor volume divided by volumetric feed rate. An inverse value of space time is dilution rate, a well known parameter in biotechnology of continuous processes. Other types of operation are also known, i.e. fed batch, repeated fed batch and recycle (Doran, 1995).

5.4 Productivity and throughput

The simplest case, a direct transformation of dye with active biomass in one reactor is considered here. The productivity of biomass during the batch fermentation can be simply defined as an increase in the biomass concentration at the point of productivity estimation from the point of inoculation divided by the process time. The productivity is shown in Figure 3 as the slope of the line. The maximum biomass productivity is achieved earlier than the maximum biomass concentration. For the process time, growth time after inoculation as well as 'dead' periods like cleaning, sterilizing and harvesting are taken into account. It should be noted that the maximum biomass productivity is not always at the point of maximum biomass concentration. In the case of batch dye decolourization/degradation, productivity should be redefined as dye degradation or dye decolourization capacity according to a specific case. For example, if the growth and enzyme production is followed by the dye degradation process in a batch reactor, the dye concentration drop from the point

of dye addition till the end of degradation process should be divided with the whole process time for growth, enzyme production, decolourization, plus dead time. For such a definition, the decolourization capacity very much depends on the initial and final dye concentrations, and the decolourization/degradation rate around the final dye concentration.

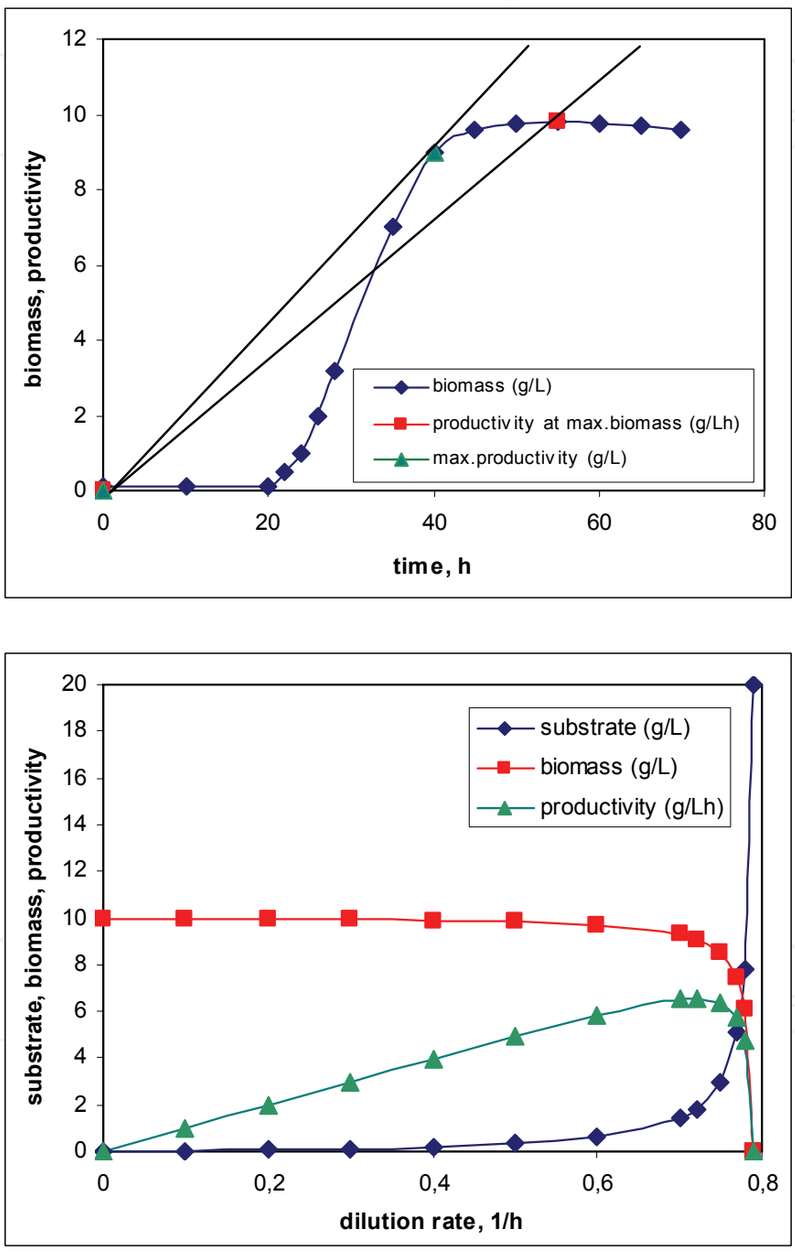

Fig. 3. Biomass productivity during batch (above) and continuous operation (below) of stirred tank reactor

The steady state concentrations during a continuous fermentation in a constant volume ideal stirred tank bioreactor are dependent on the dilution rate D = F/V, i.e. the ratio between the liquid volumetric flow rate and liquid volume in the reactor. Here, the variables like substrate, dye, enzyme, biomass and product concentrations can be considered. The critical operating point is the value of dilution rate, where the biomass and fermentation products are washed out from the reactor and the inlet stream with its components simply flows through the reactor, since there is no chemical reaction within the reactor. Figure 3 demonstrates the case of continuous biomass production for Monod growth kinetics (So = 20 g/L; $Y_{x/s}$ = 0.5; K_s = 0.2 g/L; μ_{max} = 0.8 h^{-1}). The biomass productivity here is defined as a product between biomass concentration (X) and dilution rate (D). It can be seen that it has its maximum at a certain dilution rate; therefore, the dye degradation should be controlled around this point (around 0,7 h^{-1} in our case). The dosing of dye to the reactor can be performed separately or together with the substrate inflow (F). The dye flow into the reactor with a given volume (V) must correspond to the requested dye conversion and degradation rate according to Equation 4 (Doran, 1995).

5.5 Mathematical modelling

Mathematical modelling is a useful tool for chemical engineers, which leads to better understanding of the process and reduces the number of experiments. It helps designing experiments and it is successfully used for the process regulation and control. Mathematical simulations can also be a very good enrichment for the teaching process. Briefly, it means writing down an idea of a process in the mathematical language. It consists of several steps. The system with its boundaries has to be defined first, where a chemical reaction can occur, and then the mass and energy exchange can take place. In bioremediation, the reactor is a typical system. Afterwards, the process variables and parameters have to be defined, e.g. biomass, enzyme and dye concentrations. The analysis of process dynamics gives useful information, which of the process variables considerably changes with time, or which remains constant. One of the main steps is the formulation of the mathematical model on the basis of previous steps. The type of fluid flow and various physical and chemical laws have to be considered. Equations describing mass and heat transfer, reaction rate and correlations for various coefficients are written and solved for the corresponding initial and boundary conditions. On the basis of parametric sensitivity analysis which follows, the effect of various parameters on process variables is seen and the model can be simplified. The crucial step is the verification of the mathematical model, which means a comparison of the calculated results with the model and experimental results. If the results agree within the expected deviation, the model is good and useful. If not, previous steps must be repeated by including new data (Snape et al, 1995).

5.6 Scale up

The development of a microbiological process is usually conducted in three scales, i.e. 1) laboratory scale, where a basic screening and growth conditions are investigated; 2) pilot plant, where an optimization of environmental factors is studied; and 3) plant scale, where the process is brought to an economic function. Environmental conditions involve chemical factors (e.g. media composition, pH) and physical factors (e.g. mixing, aeration, shear). They are crucial for the successful aerobic growth and enzyme production in an aerobic bioreactor, which is considered a two phase system. The transport phenomena like gas-

liquid oxygen transfer, heat transfer and mixing, as well as the chemical reactions in a liquid phase like oxygen and substrate consumption, the biomass growth and enzyme production take place simultaneously during the cultivation. On the basis of regime analysis, it must be established which of the above mentioned processes is the slowest, and therefore controls the microbial growth and enzyme production. During the transfer from the laboratory to larger scale, an optimization of this process must be considered. Historically, keeping a constant gas-liquid oxygen transfer rate in a small and large scale was mostly used, proving as a successful scale up criteria. Namely, the low rate of this process compared to other previously mentioned is characterized by low oxygen solubility in water, and can be improved with increased mixing and aeration. Usually, the geometrical similarity of both reactors was ensured and the maximum allowed impeller tip speed to avoid cell damage was taken into account. According to the above mentioned, a general scale up criteria for the microbial cultivation is to keep the optimal environmental conditions as much as possible on all scales to obtain the necessary productivity (Wang et al., 1979).

The dye degradation and/or decolourization reactions at a given enzyme activity in the solution take place in a liquid phase, and do not depend on oxygen gas-liquid mass transfer. According to the literature data, these reactions are mostly slow. The scale up of this process needs the expression of the reaction rate at a given dye concentration range, as well as the optimal pH and temperature. On the basis of the reactor type, its operation mode, rate equation and given dye conversion, the necessary degradation time in a large batch reactor of a given volume can be estimated. Similarly, the dye feed rate in a large continuous reactor can be calculated (cf. Equations 3–5).

In the case of biodegradation or decolourization in the presence of the biomass, the situation is much more complex, since the dye transport from the liquid to the active site inside the biomass has to be taken into account. Here, the degradation and/or adsorption can take place. Generally, proper mixing or fluid flow, as well as the biomass thickness can affect the dye depletion rate in the solution. For a successful scale up, a detailed investigation of the effect of the mentioned parameters on the reaction rate is necessary on the laboratory and pilot plant scale. The scale up principle may vary from case to case. Unfortunately, no research data covering this topic were found in the available literature.

5.7 Costs

Costs fall into two categories, i.e. capital costs and operating costs. Capital costs generally include initial and periodic expenses and consist of 1) design and construction, 2) equipment and installation, 3) buildings and structures, and 4) auxiliary facilities. The costs for a start up have to be taken into account in this category as well. Operating costs generally cover 1) labour, 2) equipment maintenance and parts, 3) expendable supplies and materials, 4) utilities (e.g. electricity, water, steam, gas, telephone etc), 5) ongoing inspection and engineering, and 6) laboratory analyses (Freeman, 1998).

The degradability of the dye strongly depends on its chemical structure. This fact plays an important role during the bioremediation. In addition, the fungal cultivation is done under sterile conditions, which increases the costs of the process. The dye removal efficiency is usually better with one of the chemical oxidation methods, where it can exceed 90%. The time required for oxidative decolourizations are much shorter (in minutes) compared to those needed for the adsorption or biodegradation (in hours or days) (Slokar & Majcen Le Marechal, 1998).

Practically no data on the costs of dye removal can be found. Only the evaluation of water reuse technologies for the spent dyebath wastewater containing three reactive dyes from a jig dyeing operation was found in the literature. With several methods, e.g. electrochemical oxidation, oxidation with ozone, reduction with sodium borohydride and adsorption on activated carbon, the colour removal was 78–98%, while the operating costs were estimated to be 10–94 $ per 1,000 gallons treated. Unfortunately, the dyes were toxic to the tested microorganisms and the biodegradation method was unsuccessful (Sarina, 2006).
Therefore, from this point of view, chemical methods seem for the time being more economical than the fungal bioremediation.

6. Bioreactors for fungal degradation and decolourization of dyes

A variety of reactor configurations has been used, similar to those for the fungal cultivation under submerged conditions. Gentle mixing and aeration have usually been the necessary prerequisites for a successful biomass growth and enzyme production. The immobilization of fungal mycelia also showed useful results. Batch and continuous operations were shown to be effective – both having advantages and disadvantages. Several papers have reported the repeated use of mycelia over several cycles of decolourization lasting from several weeks to a few months. Most of the studies were performed under aseptic conditions, while some were effective also during non-aseptic conditions. The toxicity of the dye highly affects the dye degradation and decolourization. Selected references from the last decade for laboratory reactors with volumes larger than 1.0 L are briefly presented below.

Type of reactor	Volume	Organism	Dye	Removal	Duration	Reference
Stirred tank	5 L	*B. adusta*	Black 5	95%	20 d	Mohorčič, 2004
Stirred tank	3.5 L	*T. versicolour*	R. Black 5 R. Red 198 Brilliant Blue R	91–99%	8 d/200 d	Borchert, 2001
Stirred tank	4.0 L	*T. versicolour*		90%	10 d	Libra, 2003
Stirred tank	1.0 L	*T. versicolour*	Poly R-478	80%	19 d	Leidig, 1999
Bubble column	1.5 L	*T. versicolour*	Orange G	97%	20 h	Casas, 2007
Bubble column	1.5 L	*T. versicolour*	Grey Lanaset G	90%	42 d	Blanquez, 2004
Packed bed	2.0 L	*Strain F29*	Orange I	95%	3.5 d HRT/60 d	Zhang, 1999
Trickle bed	1.0 L	*I. lacteus*	RO16	95%	6 d	Tavčar, 2006
Rotating discs	1.7 L	*C. versicolour*	Everzol T Blue G	80%	2 d/12 d	Kapdan, 2002
Rotating discs	1.6 L	*P. Sordida*	Basic Blue 22	80%	2 d/12 d	Ge, 2004
Rotating discs,	1.0 L	*I. lacteus*	RO16	95%	10 d	Tavčar, 2006
Rotating discs	1.0 L	*D. squalens*	RBBR, Azure B Methylene blue	99% 92% 59%	6 h 8 d 30 h	Trošt, 2010
Biofilm	1.0 L	*Fungal consortium*	RB5, AR249, RR M-3BE	70–90%	12 h/96 d	Yang, 2009
Biofilm	10.0 L	*C. versicolour*	Everzol T Blue G	82%	50 h	Kapdan, 2002
Membrane	11.8 L	*C. versicolour*	Acid Orange II	97%	1 d/62 d	Hai, 2008
Membrane	5.0 L	*P. chrysosporium*	RBR X-3B	90%	1 d/65 d	Gao, 2009

Table 5. Fungal bioreactors for degradation and decolourization of dyes

6.1 Stirred tank bioreactor

The decolourization of the diazo dye Reactive Black 5 with *Bjerkandera adusta* was conducted in a 5-L aerated stirred tank bioreactor. The fungus was immobilized on a plastic net in the form of a cylinder inside the vessel. The decolourization of the dye in an initial

concentration of 0.2 g/L from black-blue to intense yellow (95% removal) was reached in 20 days. Initially, lignin peroxidases and subsequently manganese dependent peroxidases were responsible for the decolourization (Mohorčič et al, 2004).
The white-rot fungus *Trametes versicolour* proved to be capable of decolourizing Reactive Black 5, Reactive Red 198 and Brilliant Blue R. in a 3.5-L aerated stirred tank bioreactor during a sequencing batch process. The decolourization activity was related to the expression of extracellular nonspecific peroxidases, which could be continuously reactivated by sheering the suspended microbial pellets. Under sterile conditions, 12 cycles of decolourization were performed, while under non-sterile conditions, only 5 cycles of decolourization could be achieved. One cycle lasted for 5–20 days. 91–99% of colour removal was achieved in the experiments which lasted up to 200 days (Borchert & Libra, 2001).
Various strategies for the decolourization of Reactive Black 5 with *Trametes versicolour* in a 4-L aerated stirred tank reactor with two flat-blade impellers under non-sterile conditions were compared. To obtain poor growth conditions for bacterial contamination, medium pH and nitrogen source were reduced during the cultivation of *T. versicolour* in two separate experiments. The enzyme, produced during the fungus cultivation and then isolated, was used alone for the decolourization. These three strategies were not as successful as the fourth one, where the fungus was grown on lignocellulosic solids as a sole substrate, such as straw and grain. Here, more than 90% degree of decolourization was achieved under non-sterile conditions in 10 days (Libra et al, 2003).
The mycelia of *Trametes versicolour* were aseptically encapsulated in the PVAL hydrogel beads 1–2 mm in diameter to be protected against the microbial contamination and mechanical stress. The encapsulated fungi, which were grown in a 1.0-L aerated stirred tank bioreactor under non-sterile conditions, expressed the ligninolytic enzymes which were capable of decolourizing polyvinylamine sulphonate anthrapyridone (Poly R-478). The average dye elimination of 80% was achieved in 19 days (Leidig et al, 1999).

6.2 Bubble column bioreactor

The white-rot fungus *Trametes versicolour* in the form of pellets was cultivated in a 1.5-L bioreactor, where the fluidization of biomass was achieved with a pulsating introduction of air at the bottom. The reactor was filled with separately cultivated microbial pellets, media with glucose and Orange G synthetic dye. The obtained percentage of decolourization was 97% in only 20 h. As high as 3500 AU/L of laccase was determined, while no MnP activity was detected. Better results were obtained this way compared to *In Vitro* experiments with commercial purified laccase from *T. versicolour* (Casas et al, 2007).
The batch and continuous operation mode of a 1.5-L bubble column bioreactor were used for the cultivation of *T. versicolour* in the pellet form and degradation of Grey Lanaset G metal-complex dye. A six days long batch operation was followed by a 36-day continuous operation. In both experiments, the decolourization was efficient (90%), but could not be correlated with extracellular laccase activities. The degradation occurs in several steps including the initial adsorption of the dye onto the biomass, followed by its transfer into the cells, where the degradation occurs due to the enzymes attached to the membrane (Blanquez et al, 2004).

6.3 Packed bed bioreactor

A vertical glass jar of 2.0-L working volume with an open-ended stainless wire mesh cylinder as support for mycelia growth was used for the cultivation of the fungal strain F29,

assuming to be white-rot fungus and capable of producing lignin peroxidase, manganese peroxidase and laccase. In the first 7 days of the submerged batch cultivation under aeration, the mycelium grew on the wire mesh rather than in suspension. Afterwards, the reactor was operated in a continuous mode by pumping nitrogen limited media with dye Orange II to study the decolourization process. At the retention time 3–3.5 days, the decolourization remained high (95%) for two months (Zhang et al, 1999).

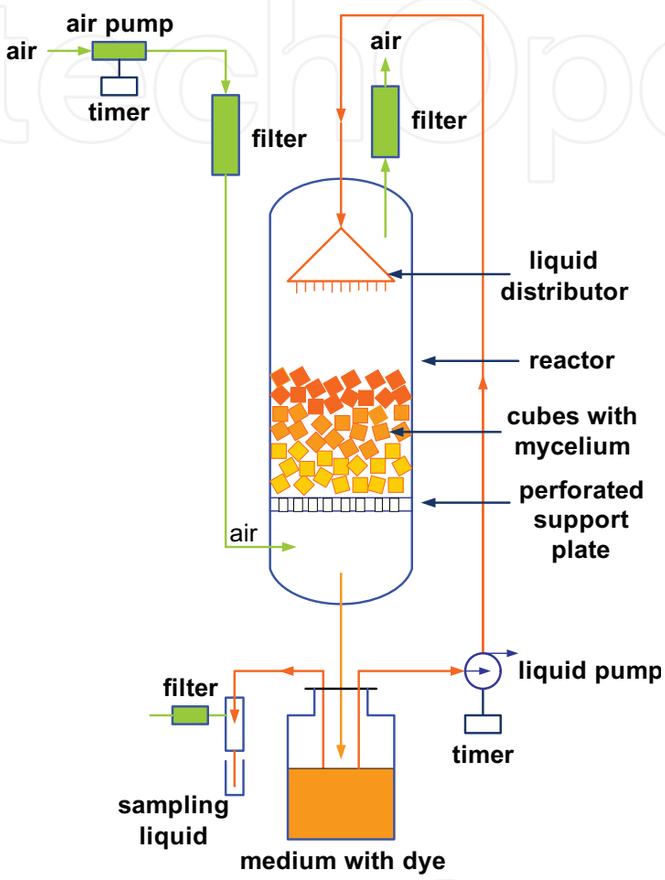

Fig. 4. Trickle bed reactor for decolourization of RO 16 with *Irpex lacteus*

The trickle bed reactor was constructed using a 10-cm ID glass cylinder, where 2-cm PUF cubes were used for the *Irpex lacteus* immobilization support. A special liquid distributor was used to uniformly distribute the liquid over the culture surface from the top of the reactor. A 2-L Erlenmeyer flask was used as a reservoir containing 1.0 L of the growth medium together with Reactive orange 16 (initial concentration 0.3 g/L), which circulated in the reactor by the means of a peristaltic pump. The reactor was also aerated through the bottom. The inoculation was done with the 10-day old fungal biomass grown on PUF. A successful decolourization due to the extracellular activities of MnP and laccases as well as the mycelium-associated laccase was performed in six days (Tavčar et al, 2006).

6.4 Rotating discs bioreactor

The biodiscs reactor consisted of 13 plastic discs with 13 cm in diameter in a horizontal cylinder with a liquid volume of 1.7 L. The rotation speed was 30 rpm. For the first three

days, the fungi *Coriolus versicolour* was cultivated in a nitrogen limited media for the biofilm formation. Then the media was replaced with fresh media with nutrients and dyestuff Everzol Turquoise Blue G. The reactor was operated in a repeated-batch mode by removing the liquid media, reloading the coloured fresh media every two days for the 12 days of operation. The decolourization efficiency was around 80% for 50–200 mg/L and 33% for 500 mg/L of initial dye concentration (Kapdan & Kargi, 2002).

The biological decolourization of Basic Blue 22 by *Phanerochaete sordida* was studied in a 1.6-L biodiscs reactor with 15 plastic discs with a 15-cm diameter at various rotational speeds 10–50 rpm. During the first 3 days, fungi were cultivated in the reactor for the biofilm formation. After that, the reactor operated in a repeated-batch mode in 2-day cycles for 12 days. A metal mesh covering the discs gave the best results, while the highest decolourization efficiency was obtained at the rotational speed 40 rpm. The TOC removal efficiency was around 80% for 50–200 mg/L and 52% for 400 mg/L of dyestuff concentration (Ge et al, 2004).

The rotating discs reactor with six 1-cm thick and 8-cm OD PUF plates was used to study the decolourization of Reactive orange 16 with *Irpex lacteus*. The liquid volume in the reactor was 1.0 L. The reactor was also aerated. First, the growth media in the reactor was inoculated with a culture homogenate and after 10 days of cultivation, when the fungus colonized the discs, the liquid in the reactor was replaced with 1.0 L of fresh medium containing 0.3 g/L of the dye. A successful decolourization due to extracellular activities of MnP and laccases, as well as mycelium-associated laccase was conducted in ten days (Tavčar et al, 2006).

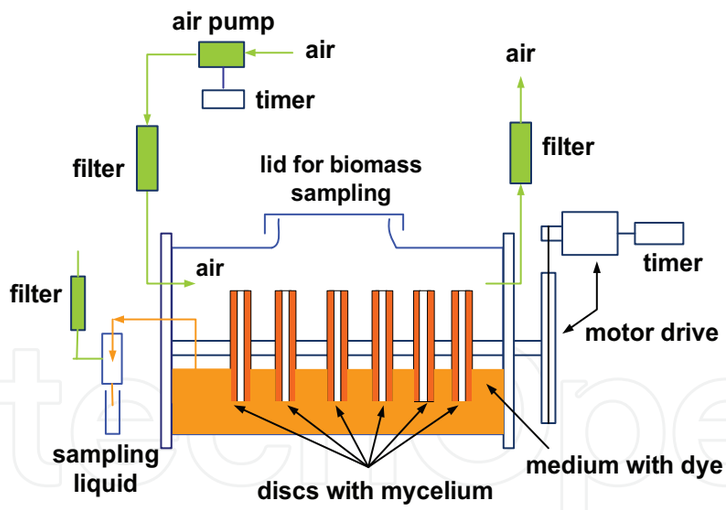

Fig. 5. Rotating discs reactor for decolourization of RO 16 with *Irpex lacteus*

Dichomitus Squalens was grown on 8.0 cm beech wood discs in a 3.0-L laboratory rotating-disc reactor (RDR) with 1.0 L of cultivation media. Three cultivations were done and the produced enzymes were used to decolourize three types of synthetic dyes, each in separate experiments: anthraquinone dye Remazol Brilliant Blue R (RBBR), thiazine dye Azure B (AB) and phenothiazine dye Methylene Blue (MB). The dye solution to obtain the initial dye concentration 50 mg/L was added to the reactor after 5 days and the following final decolourization efficiencies were obtained: 99% for RBBR after 6 h, 92% for AB after 200 h, and 59% for MB after 30 h (Trošt & Pavko, 2010).

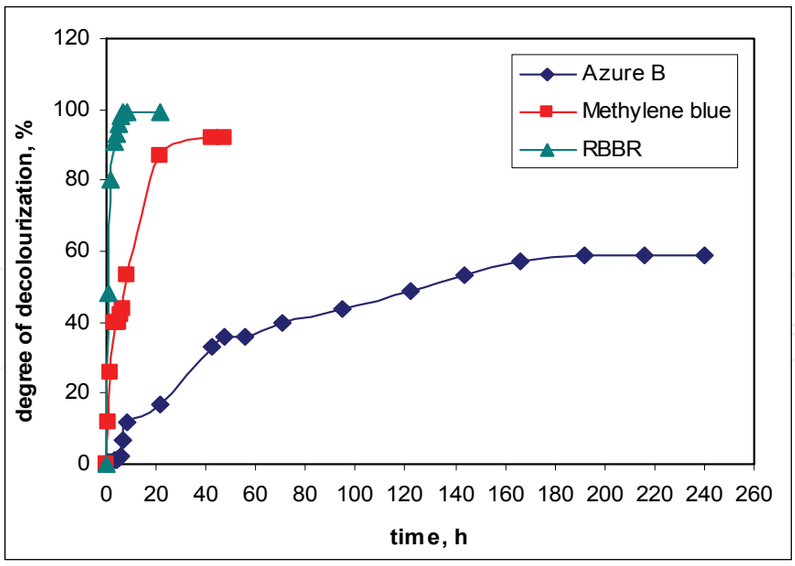

Fig. 6. Decolourization of various dyes in rotating discs reactor

6.5 Biofilm reactor

A biofilm reactor was made up of a plastic column filled with polyethylene fibre wads with a 4.5-L effective volume. 1.0 L of selected microbial consortium (obtained from rotten wood soil samples and a textile wastewater treatment plant) together with 3.0 L of growth medium were introduced into the reactor and gently aerated for the biofilm to culture under non-sterile conditions. The growth medium was replaced several times until a complete biofilm was formed. Fungi were the dominant population in the biofilm. Then, various synthetic azo dyes (Reactive Black RB5, Acid Red AR 249 and Reactive Red RR M-3BE) and textile wastewater were continuously fed into the reactor. The whole process lasted for 96 days at hydraulic retention time (HRT) of 12 h. The colour removal efficiencies were 70–80% for 100 mg/L of dye solutions and 79–89% for textile wastewaters (Yang et al, 2009).

The white-rot fungus *Coriolus versicolour* in the form of a biofilm on surfaces of inclined plates immersed in the aeration tank together with the activated sludge culture and wood ash particles as adsorbents were used for simultaneous adsorption and degradation of the textile dyestuff Everzol Turquoise Blue G. The major process variables such as dyestuff and adsorbent concentrations and sludge retention time on decolourization efficiency were studied. HRT was 50 h in all experiments. The highest colour removal efficiency was 82% at 200 mg/L of dyestuff concentration, 150 mg/L of adsorbent concentration and sludge age of 20 days (Kapdan & Kargi, 2002).

6.6 Membrane reactors

In a membrane reactor, the biocatalyst is retained within the system with a semi-permeable membrane, allowing a continuous operation with a substrate feed and product withdrawal (Lopez et al, 2002).

A cylindrical PVC bioreactor with an 11.8-L working volume was used in the study of Acid Orange II decolourization with the white-rot fungus *Coriolus versicolour*. A hollow fibre membrane module (pore size 0.4 μm) was submerged into the reactor. The system was first inoculated with the fungus and kept under aeration for 2 weeks to obtain the necessary

enzyme and biomass concentration. Afterwards, a continuous operation started by adding the nutrient sufficient synthetic wastewater with 100 mg/L of dye at HRT of 1 day under non-sterile conditions. During 62 days of successful operation, 97% of decolourization in the permeate was achieved. Later, the bacterial contamination ceased the enzymatic activity and consequently, the process efficiency (Hai et al, 2008).
A membrane bioreactor with an effective volume of 5.0 L comprised of the membrane reaction zone and hollow fibre membrane separation zone. In the reaction zone, *Phanerochaete chrysosporium* was cultivated in the form of a biofilm on the fibrous inert material. The polyvinylidene fluoride membrane (pore size 0.2 μm) was used for the separation of the permeate. The reactor was aerated during operation. After the inoculation, the reactor was operated under aeration for 8 days for the biofilm formation. Then, the dye wastewater with the dye concentration 100 mg/L was fed to the reactor, in order to achieve 24 h of the retention time. The decolourization efficiency was between 79.3% and 90.2% for the 65 days of operation, when the peroxidase isoenzyme activities were high enough. Afterwards, the biofilm retrogradation occurred and the enzyme activities decreased (Gao et al, 2009).

7. Conclusions

An enormous number of articles published in the last two decades cover the 'fungal dye decolourization'. This proves that great attention has been paid by researchers to use the lignin degrading enzymatic system of white-rot fungi for solving this serious pollution problem. A considerable amount of work in the fungal decolourization studies has been conducted on a laboratory scale to find fungal strains with effective enzymes. The main fungal enzymes have been indicated and various mechanisms have been explained, however, several studies show that unknown enzymes or mechanisms, respectively, are still present. The studies mainly cover chemically defined dyes, while the research with wastewater from dyestuff industry is rare. White-rot fungi as a group can decolourize a wide range of dyes. Nevertheless, the chemical and physical decolourization and/or degradation processes are usually faster than the processes using fungal cultures. In addition, a fungal cultivation takes place under sterile conditions, which increases the cost of bioremediation technology and additionally lowers the economics of the process. Unfortunately, there are not many results of dye degradation during the cultivation under non-sterile operation conditions available yet. Therefore, the research of screening or genetic manipulation of fungi to be more resistant, to be capable of faster dye degradation, to reach higher mineralization degree or to use dyes as sole substrates would also be of great interest.
The experiments in various types of bioreactors on a laboratory and pilot plant scale present an engineering approach to the scale up of the process, which leads to some interesting results. From the economical point of view in general, the process should be fast and effective. There are several descriptions of degradation kinetics with isolated enzymes and a few with the whole mycelia, but for the industrialization of fungal bioremediation, more attention should be paid to the degradation kinetics studies. The studies of pilot plant reactors with volumes 10–100 L for the transfer to a larger scale could be more intense. There is a lack of comparative data to indicate the best reactor configuration. On the other hand, the research in the last decade shows that the membrane reactors have an interesting potential. There is practically no data about the bioremediation costs; it would be very interesting to compare this promising technology with alternative processes for the treatment of effluents with synthetic dyes.

Moreover, the mathematical modelling of the decolourization process has not gained such significance here, as it has in other fields of biotechnology.

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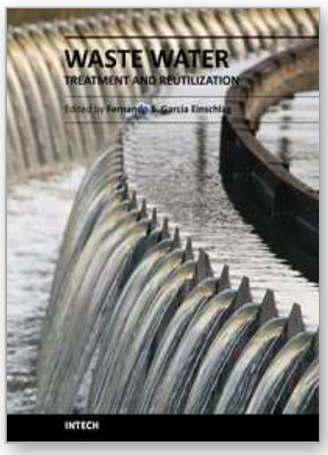

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The steady increase in industrialization, urbanization and enormous population growth are leading to production of huge quantities of wastewaters that may frequently cause environmental hazards. This makes waste water treatment and waste water reduction very important issues. The book offers a collection of studies and findings concerning waste water treatment, minimization and reuse.

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