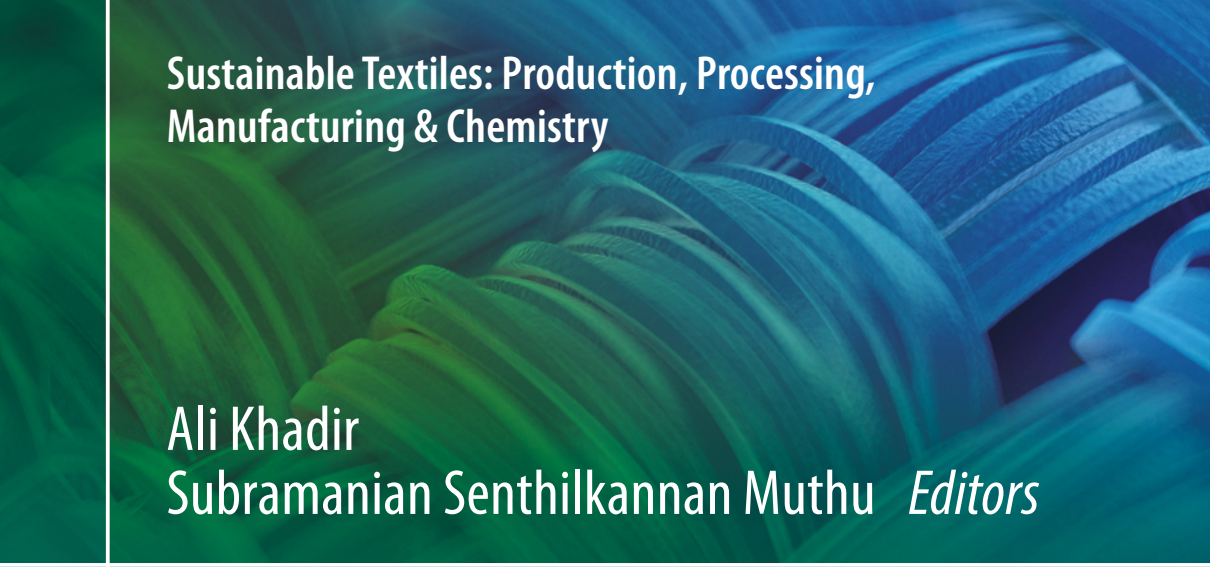

Sustainable Textiles: Production, Processing, Manufacturing & Chemistry

Ali Khadir
Subramanian Senthilkannan Muthu *Editors*

Biological Approaches in Dye-Containing Wastewater

Volume 1

Springer

Sustainable Textiles: Production, Processing, Manufacturing & Chemistry

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Editors

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ISSN 2662-7108 ISSN 2662-7116 (electronic)
Sustainable Textiles: Production, Processing, Manufacturing & Chemistry
ISBN 978-981-19-0544-5 ISBN 978-981-19-0545-2 (eBook)
https://doi.org/10.1007/978-981-19-0545-2

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The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

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Overview of Biological Technologies for Azo Dye Removal

L. P. Silva Júnior, I. R. M. Câmara, A. B. S. da Silva, F. M. Amaral, F. Motteran, B. S. Fernandes, and S. Gavazza

Abstract The textile industry segment has been continuously expanded and is one of the most expressive consumers of chemical products, dyes, and water. As a result, the sector is responsible for generating a large amount of industrial wastewater. These effluents are highly toxic and potentially carcinogenic and mutagenic. Azo dyes are widely used in the textile industry; thus, they are commonly found in its wastewater. This chapter approaches the main processes of azo dyes removal and biodegradation, highlighting the most used and effective reactor configurations. While anaerobic reactors are most efficient for combined color and organic matter removal, aerobic reactors are required to mineralize aromatic amines, byproducts of azo dye degradation. Therefore, combination of anaerobic and aerobic reactors is desired. The following topics are also covered: the influence of operational parameters; effectiveness of applying one- or two-stages (aerobic and anaerobic) processes; and metabolism, stoichiometry, and byproduct formation. Sequencing Batch Reactors (SBR) are the most used one-stage system, showing good removal efficiencies. Upflow Anaerobic Sludge Blanket (UASB) and Expanded Granular Sludge Blanket (EGSB) reactors were effectively used as anaerobic reactors, while different biofilm aerobic reactors showed excellent performance on removing aromatic amines.

Keywords Textile wastewater · Effluent treatment · Recalcitrant effluent · Aromatic amines · Environmental contamination · Biodegradation · Anaerobic–aerobic degradation · Reactor configuration · Two-stage systems · Metabolism of microorganisms

1 Introduction

In 2020, the global textile market was estimated at US$1000 billion, with expected growth given in Compound Annual Growth Rate (CAGR) of 4.4% from 2021 to

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A. Khadir et al. (eds.), *Biological Approaches in Dye-Containing Wastewater*, Sustainable Textiles: Production, Processing, Manufacturing & Chemistry,
https://doi.org/10.1007/978-981-19-0545-2_1

2028. Pacific Asia alone accounted for over 47% of the global revenue in 2020 [48]. China is the world leader in the production and export of textile and clothing raw materials, followed by the United States, which is considered the main producer and exporter of raw cotton, in addition to being the largest importer of raw textiles and clothing [83]. India is considered the third-largest textile manufacturing industry [83]. The largest textile producer countries in the world, by region, are the United States in North America, Brazil in Central and South America, Germany in Europe, Saudi Arabia in the Middle East & Africa, and China in the Asia Pacific [48].

The textile segment is one of the most expressive consumers of chemical products, dyes, and water. As a result, this sector is responsible for discharging a large amount of highly toxic and potentially carcinogenic and mutagenic wastewater [11, 47]. This wastewater is characterized by high contents of organic matter, suspended solids, chemical oxygen demand (COD), salinity, and frequently, sulfate. Low biochemical oxygen demand (BOD) and COD ratio, together with intense color, complement the composition and give so much challenge to treat this wastewater [29, 121].

The most used dyes in the industries, among the organic dyes, are of the azo type. Characterized by the chromophore azo bond (-N = N-) in the molecular structure, they present toxic characteristics. In addition, their biodegradation leads to the formation of aromatic amines, which are often equally or more toxic to the environment than the dye itself [2, 5, 90].

In order to treat the wastewater from the textile industry, physicochemical processes have been frequently studied and applied [84, 98, 99, 115]. However, these technologies present high energy demand and maintenance cost and may not mineralize the toxic compounds, which makes them less viable for low-income regions. These treatment methods also generate large amounts of non-inert chemical sludge, which increases the cost of treatment and disposal and makes the process often not feasible for small producers and small-scale industries [60].

In this context, biological processes are interesting alternatives because they are low-cost and ecologically friendly technologies, removing color and reducing toxicity, frequently achieving compound biomineralization [121]. Among the existing technologies, the following stand out: anaerobic processes [4, 18, 39], aerobic processes [43, 51], and more recently, since the 90 s, the combination of anaerobic and aerobic processes [9, 121] has been studied applying one-stage [7, 8, 16] and two-stage systems [1, 12], 36; [58, 87, 94, 112]. These solutions become interesting, since the biological degradation process of azo dyes starts in an anaerobic environment for the reductive cleavage of azo bonds, leading to color removal. Nonetheless, the byproducts from azo dye degradation are often hazardous recalcitrant compounds that are better degraded in an aerobic environment [17].

Finally, this chapter elucidates the main technologies for the biological treatment of effluents from the textile industry, combining aspects of engineering, chemistry, and biological process.

2 Anaerobic Reactors

2.1 Color Removal of Azo Dye Textile Effluent in Anaerobic Reactors

The mineralization of azo dyes begins with the reductive cleavage of the azo bond, which occurs more easily under anaerobic conditions due to the low redox potential (≤ -50 mV) [11, 79]. This reaction occurs with the aid of the enzyme azoreductase [29]. In this process, breaking the double bond between nitrogen atoms involves the transfer of 4 electrons, as shown in Fig. 1.

In the reductive cleavage process, the azo dye acts as the electron acceptor, due to the electron withdrawing capacity of the azo bond. The presence of an electron donor is required and reducing equivalents, biologically formed by the fermentation of easily degrading organic matter, commonly perform this role [2]. The addition of co-substrates is encouraged in order to increase the amount of reducing equivalents available in the environment [28, 29, 103]. Nevertheless, their use in excessive concentrations can cause unwanted effects on the system, such as decreased color removal efficiency [22].

The use of redox mediators can improve the dye degradation process [29, 102]. Since in anaerobic mixed cultures, the production of reducing equivalents is not a limiting factor, the electron transfer to the azo bond can become the drawback. In this context, redox mediators are chemical compounds that have low molecular weight and act as accelerators of azo bond cleavage. First, the mediators are enzymatically reduced using the available organic matter as the electron donor. Then, there is a chemical transfer of the captured electrons to the azo dye, resulting in the recovery of the mediator in its oxidized form [19, 20].

Given the electrophilic characteristic of the azo bond ($-N = N-$), that is, its high electron affinity, azo dyes are better degraded under oxygen-free conditions [93, 111]. This happens because oxygen in its free form (O_2) or in its combined form competes with the dye, as an acceptor, for the electrons of the organic matter. As a result, organic matter removal may be high, but color removal may be low [2]. The presence of color in textile effluents is not only an aesthetic problem but also an

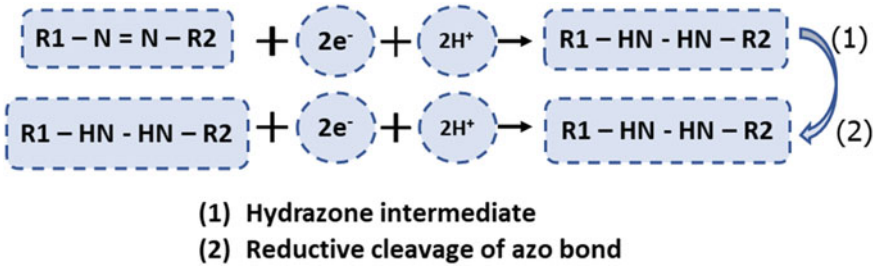

Fig. 1 Electron requirements for reactions steps of reductive cleavage of azo bonds

environmental one, with negative impacts on water bodies, inhibiting photosynthesis in water. Thus, high color removal efficiency is desirable and the anaerobic reactors are the most used for this purpose.

2.2 *Configuration of the Anaerobic Treatment Systems*

Anaerobic reactors are used under different configurations, such as anaerobic filter, packed-bed, anaerobic rotating disc, fluidized bed reactor (FBR), structured bed reactor (SBR), UASB (Upflow Anaerobic Sludge Blanket) reactor and expanded granular sludge bed (EGSB) reactor [121]. This chapter will mainly focus on the performance of UASB reactors for textile wastewater treatment, since it is the most used configuration with this purpose. Figure 2 shows a didactic schematic of the most used anaerobic reactors.

The UASB reactor is a widely used anaerobic reactor configuration for color and organic matter removal in textile wastewater [11, 18, 20, 28, 103]. Initially designed for treating industrial effluents at mesophilic temperatures (between 20 and 45 °C), it is also used for treating domestic effluents [40]. In a UASB reactor, the effluent passes through a dense blanket of anaerobic sludge, in an upward flow, reaching a three-phase separation unit (solid/liquid/gas) [71]. Due to the high biomass concentration in the reactor, it tolerates high organic loads and is widely used in the treatment of complex effluents [29]. When treating highly toxic compounds, such as azo dyes, these reactors. can reach high treatment efficiency, ensuring stability [2, 20, 28, 103].

As a result of low sludge production, the generation of energy in the form of biogas, and its low costs in comparison to aerobic treatments, the UASB reactor is considered a sustainable technology. Furthermore, it is considerably versatile and goes from small to large scales [70]. Mechanical agitation is not used in UASB reactors to avoid disaggregation and granule shear. Additionally, biogas production or effluent recycling can promote contact between biomass and effluent.

The study on textile effluent treatment in anaerobic reactors has advanced during the 1980s and 1990s [9]. Nonetheless, isolating these azo dye-degrading cultures remains a challenging task. Still, studies with anaerobic reactors inoculated with mixed cultures have been widely used as a single treatment for textile effluents during the 1990s and 2000s [20, 22, 28, 103, 122].

2.2.1 Influence of Operational Conditions on Color Removal from Azo Dye Textile Effluent by UASB Reactors

Modifying the operational settings of the reactor can optimize color removal efficiency and should be chosen with caution to achieve a more robust treatment of complex effluents. When high color removal efficiencies are the target, the optimal

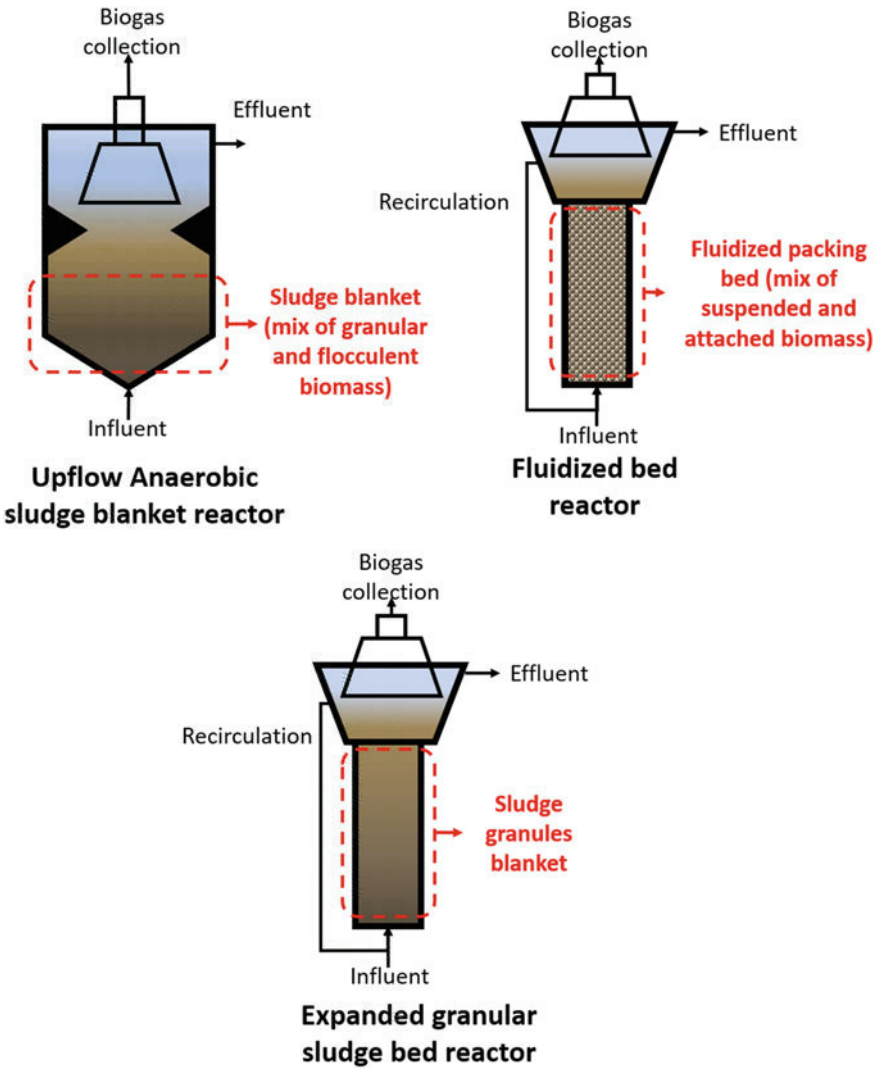

Fig. 2 Common anaerobic reactor configurations used for degradation of azo dyes

conditions necessary for dye cleavage must be considered, such as hydraulic retention time (HRT), the choice of co-substrate, the use of a redox mediator, process temperature, initial dye concentration, and others [121].

The higher the hydraulic retention time (HRT), the longer the biomass will be in contact with the effluent. When HRT is increased, the organic load applied to the reactor decreases, and the overall removal efficiency is expected to increase. Razo-Flores et al. [103] assayed 160 mL UASB reactors with 75 mg/L (0.25 mM*) of the azo dye azodisalicylate, using glucose as co-substrate. An increase in HRT from 8

to 16 h and to 26 h led dye removal efficiencies to increase from 63.4% to 69.1% and to 88.9%, respectively. Nonetheless, a decrease in HRT below optimal values can lead to a significant decrease in removal rates, but when combined with other parameters it can yield different results. In the study of [20], using 100 mg/L of the azo dye Acid Orange 7 (AO7) (0.29 mM*) in a 1.3 L UASB reactor, the decrease in HRT from 6 to 4 h and to 2 h decreased color removal efficiency from 98 to 90% and to 70%, respectively. After adding 3 μM (1.1 mg/L*) of the redox mediator Anthraquinone-2,6-disulfonate (AQDS), color removal was above 90% for all HRTs. The configurations used by both authors can be seen in Table 1.

The use of easily-degradable organic matter as electron donor is indicated to facilitate azo bond cleavage. Different co-substrates provide different effluent color removal rates. Table 1 shows different configurations of the use of co-substrates. Chinwetkitvanich et al. [22] used tapioca starch as a co-substrate in UASB reactors, at 200 and 500 mg/L, when treating real effluents. Color removals of 58% and 57%, respectively, of the effluent containing blue dye, and 52% and 56%, respectively, of the effluent with red dye were achieved, suggesting these concentrations should be used for the decolorization of 150 color SU (space units).

Donlon et al. [28] inoculated two separate 160 mL UASB reactors with anaerobic granular sludge and used either glucose (1.3 g COD/L) or a mixture of volatile fatty acids (VFAs)—acetate, propionate, and butyrate—(1.5 g COD/L) as co-substrate. The authors reported the removal of 95.1% and 91.2%, respectively, of 0.35 mM (101 mg/L*) and 0.18 mM (52 mg/L*) of influent Mordant Orange 1 (MO1). When no co-substrates were received, the reactor failed after 50 days. The use of 10 g/L of dextrin (19.8 mM*) and 5 g/L of peptone (72 mM*) as co-substrate in 500 mL serum bottles by [11] treating 300 mg/L of the reactive azo dyes Black 5 (0.30 mM*), Red 2 (0.49 mM*), Red 120 (0.22 mM*), Yellow 3 (0.51 mM*), Yellow 15 (0.47 mM*) and Yellow 17 (0.44 mM*), resulted in color removal between 77.8% and 97.1%.

Redox mediators can be used as catalysts for the reductive cleavage process of azo dyes. Figure 3 shows a schematic of the azo bond cleavage process combined with the use redox mediators. Their use improved the decolorization efficiency of a synthetic effluent in the study performed by Dos Santos et al. [30]. The authors reported the speed up in the decolorization rate of 0.3 mM of azo dyes (Reactive Red 2 (RR2) (185 mg/L*), AO7 (105 mg/L*), or MY10 (110 mg/L*), by up to eightfold compared to the mediator-free bottles in an EGSB reactor, with all three redox mediators (RM) used, Anthraquinone-2,6-disulfonate (AQDS), anthraquinone-2-sulfonate (AQS) and riboflavin (vitamin B2). Cervantes et al. [20] also reported a decolorization increase from 70% to above 95% of 100 mg/L of azo AO7 (0.29 mM*), with different doses of ADQS, 3, 10 and 30 μM (1.1, 3.7 and 11.0 mg/L*) in a UASB reactor, even at low HRTs (2 h). Similar results were reported by [4], treating a synthetic effluent with 0.06 mM (65 mg/L*) of the azo dye Direct Black 22 (DB22) in a UASB reactor, using 0.012 mM of Lawsone (2 mg/L*) and Riboflavin (45 mg/L*) as redox mediators. The use of Lawsone led to 90% of effluent decolorization and 87% of Riboflavin decolorization. However, when tested in real textile wastewater, the authors reported color removal of only 23% with Lawsone (when used with Sucrose as electron donor) and

Table 1 Important operational parameters of different anaerobic systems used for degradation of

Reactor type	Azo dye and concentration[a]	COD (mg/L)	HRT (hours)	Electron donors	Redox mediators	Color removal (%)	COD removal (%)	Reference
160 mL serum flasks	MO1 (0.18–0.35 mM)	910–1420	0.31–0.34 (days)	Acetate, propionate and butyrate, or glucose	–	91.8–95.1	81.8–86.6	Donlon et al. [28]
160 mL glass UASB bioreactors	Azodisalicyate (25–75 mg/L)	1000–3000	8, 16, and 26	Acetate, propionate and butyrate or glucose	–	72.8–98.8	84.1–94.8	Razo-Flores et al. [103]
500 mL serum flasks	RBk 5; RY 15; RR 2; RR 120; RY 3; RY 17 (300 mg/L)	–	Batch assays	dextrin, peptone	–	77.8–97.1	–	Beydilli et al. [11]
120 mL serum flasks	AO 7; AR 266; AY 137; AY 159; BR 23; DBk 19; DBk 22; DBl 53; DBl 71; DR 79; DR 81; DY 4; DY 12; DY 50; MO 1; MY 10; RB 5; RO 14; RO 16; RR 2; RR 4; RY 2 (0.3 mM (100–300 mg/L))	–	Batch assays	Acetate, propionate and butyrate	–	73–100	–	Van der Zee et al. [122]

(continued)

Table 1 (continued)

Reactor type	Azo dye and concentration[a]	COD (mg/L)	HRT (hours)	Electron donors	Redox mediators	Color removal (%)	COD removal (%)	Reference
1.3L lab-scale UASB	AO7 (100 mg/L)	–	2, 4, and 6	Acetate, propionate and butyrate	anthraquinone 2,6-disulfonate	>90	79–86	Cervantes et al. [20]
EGSB[d]	RR 2, AO 7, MY 10 (0.3 mM)	–	6	Acetate, propionate and butyrate mixed with glucose	Anthraquinone-2,6-disulfonate (AQDS), anthraquinone-2-sulfonate (AQS), riboflavin (vitamin B2), and cyanocobalamin (vitamin B12)	–	–	Dos Santos et al. [31]
100 mL glass serum flasks	DB 22 (0.06 mM)	1412–1599	Batch assays	Sucrose and ethanol	Lawsone, Riboflavin	83–93	24–69	Amorim et al. [4]
100 mL glass serum flasks	Dye mixture (317–450 mg Pt–Co/L)	1351–1530	Batch assays	Sucrose and ethanol	Lawsone, Riboflavin	23–38	26–65	Amorim et al. [4]
1.1L UASB	RR2 (40–400 mg/L)	> 1000	12	Glucose	Humic substances	67–98	36–73	Cervantes et al. [18]
3.5L UASBR[e]	DB 22 (0.06 mM)	1078	24	Ethanol	-	68 ± 5	77.7 ± 9.2	Florencio et al. (2021)

[a]Abreviations - First letter: A = Acid; B = Basic; C = Ciba; D = Direct; M = Mordant; R = Reactive. Second letter: O = Orange; Y = yellow; R = Red; Bk = Black; Bl = Blue; N = Navy
[b]WW = Wastewater
[c]WWTP = Wastewater Treatment Plant
[d]EGSB = Expanded Granular Sludge Bed
[e]UASBR = Upflow Anaerobic Structured-Bed Reactor

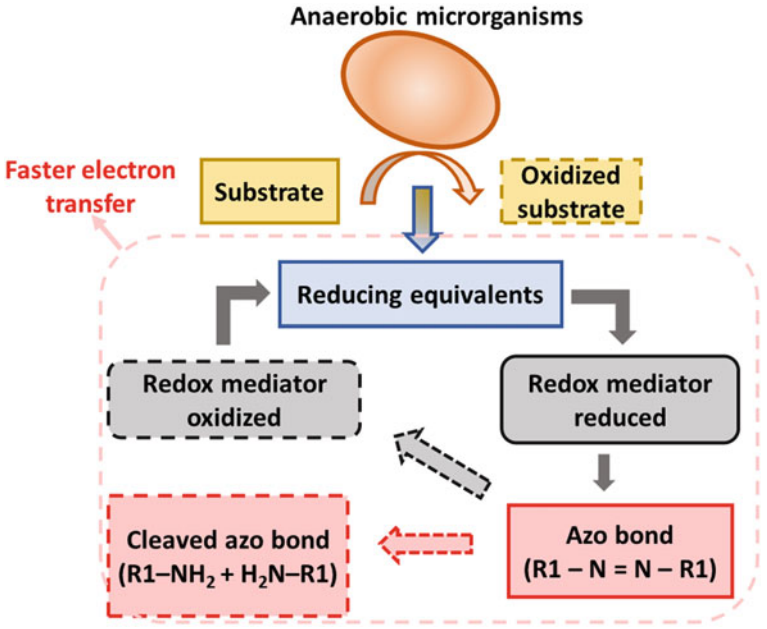

Fig. 3 Flowchart of the anaerobic reductive cleavage of azo bonds process aided by redox mediators

26% with Riboflavin (with ethanol as electron donor). The operational parameters of the reactors used by the authors are referred to in Table 1.

Process temperature influences the metabolism of the bacterial community, since it is directly related to the kinetics of the enzymatic reactions. Although most of the reactors are operated at a mesophilic temperature range, Dos Santos et al. [30] reported faster decolorization of the azo dyes RR2, AO7, and MY10 under thermophilic conditions (55 °C) compared to mesophilic conditions (30 °C), using EGSB reactor and AQDS as redox mediator. Furthermore, no lag phase was observed for the thermophilic conditions, suggesting this temperature range as advantageous over the mesophilic, for the tested conditions.

The initial concentration of the dye also has a great influence on its removal efficiency. This is mainly due to the toxicity characteristics of the dyes and the ability of microorganisms to overcome the toxicity, making some cultures more tolerant than others. High initial concentrations of azo dyes can inhibit community growth, leading to a decline in reactor efficiency. Beydilli et al. [11] analyzed the degradation of azo dye RR2 at initial concentrations of 50, 300, 500, 1000, and 2000 mg/L (0.08, 0.49, 0.81, 1.63, and 3.25 mM*) in 500 mL UASB reactors and verified that at the concentrations of 50 and 300 mg/L, complete color removal was achieved in the first 1 and 122 h of incubation, respectively, without inhibitory effects. Nevertheless, color removal was not completed within 400 h for the other concentrations, and there was an inhibition in the methanogenesis process. The different initial dye concentrations used by the authors are shown in Table 1.

Some characteristics of the azo dye itself must also be observed aiming to improve color removal efficiency. Van der Zee et al. [122] used 0.3 mM (100–300 mg/L) of 20 different kinds of azo dyes, including mono, di, tri, and tetra azo dyes (which have 1, 2, 3 or 4 azo bonds, respectively). The authors demonstrated that the rate of dye decolorization was not dependent on its molecular weight. On the other hand, chemical groups on the dye structure do influence color removal. Kulla et al. [69] demonstrated that the sulfonic group in azo dye molecules can decrease removal efficiency: (a) by hindering the enzymatic attack of the azoreductase, (b) by destroying the ability of the molecule to induce this enzyme or, (c) by its electronegativity, "shielding" the molecule from enzymatic attack.

2.2.2 Use of Other Anaerobic Systems on the Treatment of Azo Dye Textile Effluents

Fluidized bed reactor (FBR) and the expanded granular sludge bed (EGSB) reactor, originating from UASB reactors, have also shown good performance on decolorizing azo dye effluents.

The configuration of fluidized bed reactors allows the use of alternative support materials, such as sand, activated carbon, pumice stone, kinasite, tire scraping, glass beads, among others. Thus, the biomass adheres to the support material and is distributed in the reactor, increasing the contact surface between wastewater and microorganisms [10, 62, 85, 86, 92]. Maintaining the stability of this type of reactor in practice is more complicated than the UASB reactors, because it is necessary to control aggregate formation in the biofilm. This is performed adjusting the upflow speed of the liquid, size, and density of the dispersed particles of the influent wastewater, aiming to avoid the disaggregation of the biofilm formed in the support media [70]. Despite that, Haroun et al. (2009) reported color (65%) and soluble organic matter (98%) removal from a real textile effluent using FBR with activated carbon as support material, 0.6 g/L of glucose (3.3 μM*) as co-substrate, and 12 h HRT. Cirik et al. [24], in a study using a synthetic effluent of the azo dye Remazol Brilliant Violet 5R at concentrations from 100 to 200 mg/L (0.14–0.27 mM*), reported the improvement in FBR removal efficiencies. Using activated carbon as support medium color and organic matter removal, using glucose and ethanol as co-substrate, ranged from 60 and 76% to 75% and 99%, respectively.

In the Expanded Granular Sludge Bed (EGSB), granular biomass is used as inoculum. The granular bed has an expanded section, allowing greater contact between the influent wastewater and the microbial biomass. This configuration allows higher upflow speed than FBR, which can be boosted by effluent recirculation, improving the removal efficiency of toxic and recalcitrant compounds [70]. Dos Santos et al. [32] used EGSB reactor for the treatment of 1.35 g/L of the azo dye RR2 (2.19 mM) and reported 91% of color removal and 62% of organic matter removal. This shows that configurations of non-conventional or hybrid anaerobic reactors can be interesting alternatives in the treatment of. wastewater containing azo dyes, since they provide excellent results in both organic matter and color removal.

2.3 *Byproducts of Azo Dye Reductive Cleavage and the Challenges for the Anaerobic Treatment*

It is well established in literature, as demonstrated in the previous sections, that the cleavage of azo dyes requires an environment with low redox potential. Thus, anaerobic reactors are the most suitable for that job. Nonetheless, despite removing color from the effluent, the reductive cleavage of azo dyes results in compounds with at least one radical with an amino group (NH_2) [90, 116]. These byproducts are colorless compounds that are not metabolized in an anaerobic environment, due to the electronic stability of the benzene ring, which makes bond breakage hard and less energy-yielding for microbial cells in these environments [38, 42]. It is necessary to remove aromatic amines from the effluent, since they are even more toxic than the dye that produced them [2, 36, 37].

Kulla et al. [69] studied the azo dye Orange I, demonstrating that the sulfonic group present in the structure influences dye mineralization. The *Pseudomonas* strains K22 and KF46 used in the anaerobic degradation of the dye conducted the reductive cleavage of Orange I, partially removing the color from the synthetic effluent. However, one of the reductive cleavage byproducts, sulphanilic acid, was not metabolized, accumulating brownish-colored substances instead of releasing CO_2. The authors discuss that the non-utilization of the metabolite by microbial strains may occur because of the low cell permeability of the sulfonated molecules and their high electronegativity, which hinders enzymatic attack. Additionally, the increase in the remaining intracellular sulphanilic acid may have caused the destruction in the ability of the molecule to induce azoreductase, causing a decline in the cell ability to degrade the dyes.

The poor performance of anaerobic environments in degrading aromatic amines was also reported by Pereira et al. [96], who studied two simple aromatic amines, aniline and sulphanilic acid, in UASB reactors in the presence of nitrate and nitrite. Aniline was consumed with nitrate, but in a combination of nitrate and nitrite, aromatic amines ended up chemically reacting and forming other aromatic compounds. The amines formed had higher molecular weights and were more difficult to degrade and hence, more recalcitrant. Although these results are promising and similar studies have been reported on the anaerobic degradation of aromatic amines [80, 117], biological processes using oxygen as electron acceptor are still energetically favorable.

Currently, studies performed in anaerobic reactors focus more on increasing color removal efficiency along with the removal of azo dye byproducts and nutrients (nitrogen, phosphorus, carbon, sulfur), and on the generation of energy by the use of reactors combined with electrodes [3, 18, 35, 39, 44, 64]. Nonetheless, most of the reductive cleavage byproducts do not exempt aerobic treatment, since they are persistent in environments with low redox potential [59, 96]. Further studies using aerobic reactors for the complete mineralization of azo dyes have been conducted, which is discussed in the next session.

3 Aerobic Reactors

3.1 Color Removal of Azo Dye Textile Effluent in Aerobic Reactors

Azo dyes are not removed by conventional aerobic treatment systems nor rapidly degraded, since oxygen is the preferred electron acceptor [53, 61, 116]. Nevertheless, there have been indications that aerobic decolorization can occur in the presence of certain enzymes. Dye degradation under aerobic conditions is catalyzed mainly by azoreductase enzymes, but it also occurs in the presence of Nicotinamide Adenine Dinucleotide Hydrogen-Dichlorophenolindophenol reductase (NADH-DCIP reductase), malachite green reductase (MG reductase), and oxidative enzymes like lignin peroxidase and laccase [110]. Studies indicate that the action of some aerobic microorganisms combined with the enzyme azoreductase improves the kinetics of the azo dye degradation process [72]. In addition, some microorganisms that can aerobically decolorize azo dyes by the catalysis of aerobic or oxygen-insensitive azoreductases have been isolated [63, 82, 89]. Factors such as dye concentration, enzyme concentration, temperature, and intermediate complex formation rate also influence and contribute to the reduction of azo dyes [110].

It has also been observed that aerobic processes for the treatment of effluents that contain azo dyes are ineffective in most systems containing textile industry wastewater, although few microorganisms can partially or completely degrade dye molecules. However, the products formed during the anaerobic process, aromatic amines, can be demineralized or decomposed by the aerobic process. The aerobic treatment is essential for the degradation of the byproducts of the anaerobic degradation, and the removal of the toxicity generated by the production of aromatic amines.

3.2 Configuration of Aerobic Treatment Systems and Strategies to Enhance Color Removal

Several studies using bacteria, fungi, and algae capable of reducing azo dyes have been reported [27, 46, 105]. Bacteria are widely used for decolorizing azo dyes due to their high activity, extensive distribution, and strong adaptability [29, 95]. Nevertheless, the decolorization intermediates, such as aromatic amines, can inhibit the activity of a large number of bacteria [101]. On the other hand, fungi have a strong ability to degrade complex organic compounds by producing extracellular ligninolytic enzymes, including laccase, manganese peroxidase and lignin peroxidase, drawing attention to fungi in recent years [46, 101]. So far, some species, such as *Pleurotus ostreatus*, *Pichia* sp., *Penicillium* sp., and *Candida tropicalis*, have been confirmed to discolor azo dyes by adsorption or degradation [6, 55, 101].

Buitrón et al. [15] evaluated the aerobic degradation of the azo dye Acid Red 151 (AR151) by a sequencing batch biofilter packed with a porous volcanic rock, reaching up to 99% of color removal from an initial concentration of 50 mg/L (0.11 mM*) of AR151. Coughlin et al. [26] isolated two bacterial strains capable of reducing the azo bond of dyes AO7 and AO8, at 50 mg/L (0.14 mM*). One of the strains used both dyes as its only source of carbon, nitrogen, and energy, while the other could only reduce the azo bond when co-substrates (0.05% glucose and 0.2% NH_4SO_4) were present. Some azo dyes can be degraded by aerobic bacteria in the presence of suitable co-substrates [103]. The aerobic degradation of a simple azo compound (Orange Carboxy II) by *Flavobacterium* sp. was reported by [68].

Tan et al. [118] evaluated the aerobic decolorization and degradation of azo dyes both by suspended growing cells and immobilized cells of a newly isolated yeast strain, LH-F. The effects of different parameters on the decolorization of Acid Red B by growing cells of strain LH-F1 were investigated, including initial dye concentration (50–300 mg/L), concentrations of glucose (2–14 g/L), and ammonium sulfate (0.5–3.0 g/L), inoculation size (2%–10%, v/v), temperature (20–40 C), and pH (3–9). More than 90% of all six azo dyes were decolorized by strain LH-F1, and more than 98% of Acid Red B was removed within 10 h when the initial dye concentration was 50 mg/L.

The activated sludge system is the most promising aerobic system for treating wastewater containing azo dyes because it can be operated for a long period without too many concerns about the elimination of specific microbial strains from the treatment systems and frequent inoculation [74]. Aerobic granular sludge has attracted great attention for wastewater treatment containing azo dyes, since its compact structure and bead size allow them to resist the shock of toxic compounds and harsh environmental conditions. The more diverse the microbial population, the greater will be the removal of pollutants. Aerobic granules can be easily formed with azo dyes at low concentrations, such as 50 mg/L^{-1}, mixed with other biodegradable carbon sources, such as glucose, starch or ethanol [74]. Mata et al. [78] reported better granulation results with the addition of 20 mg/L of the azo dye AR14 to a synthetic textile effluent, using starch as carbon source, and organic matter concentration around 1000 mg/L^{-1}, using a batch reactor system for 62 days. Ibrahim et al. [52] developed granules capable of treating real textile wastewater from a mixture of sterilized activated sludge and bacterial species isolated from textile sludge.

Furthermore, during aerobic decolorization processes, the breakdown of compounds could be further degraded by monooxygenase and dioxygenase catalysis, which would induce the incorporation of O_2 into the aromatic ring of organic compounds before ring fission, as cited by [107]. Some azo dyes could be decolorized or even mineralized by certain microorganisms under aerobic conditions. Therefore, compared to the conventional two-stage system, aerobic processes with selected microbial strains become simple and economical alternatives for the treatment of textile effluents containing azo dyes. The conventional process of activated sludge treatment is one of the important aerobic treatment methods for azo dye degradation. In addition, it is often an effective and highly economical method that reduces organic pollutants present in several wastewaters. Nevertheless, the activated sludge

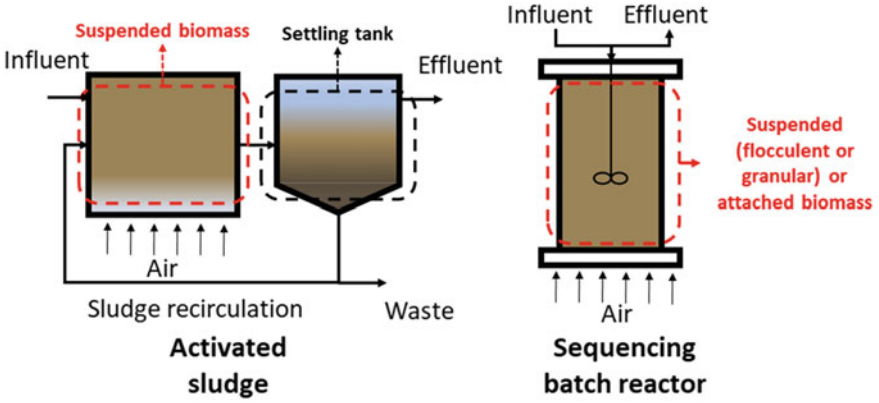

Fig. 4 Schematic of the main reactor configurations used for aerobic degradation of azo dyes

system used for aerobic azo dye treatment proved to be an ineffective process in most cases, since the degradation rate of azo bonds is significantly lower in the presence of aerobic microorganisms compared to the anaerobic process. Figure 4 shows a schematic of the activated sludge and sequencing batch reactors.

Different microbial cultures were isolated aiming at the discoloration and degradation of dyes under different conditions, such as anaerobic, microaerobic, aerobic, or alternating anaerobic and aerobic conditions. The consortia have proven to be more efficient in treating textile effluents containing azo dyes [81, 123]. Table 2 shows some types of aerobic effluent treatment systems containing azo dyes.

3.3 Removal of Azo Dye Textile Effluent Byproducts in Aerobic Reactors

The selection of the best option of biological treatment for the bioremediation of a specific type of industrial effluent is a difficult task, given the complex composition of this effluent. An efficient combination for the removal of azo dyes is the use of systems or processes in two or more stages, and the choice depends on effluent composition, dye characteristics, cost, toxicity of the degradation products, and future use of the treated effluent [113].

Despite the ineffectiveness of aerobic reactors in azo dye treatment, its use is studied as an anaerobic additional post-treatment for the mineralization of their byproducts. The most energetically favorable path for the biodegradation of aromatic amines is by introducing hydroxyl groups into the aromatic ring by oxygenase-like enzymes, destabilizing the ring's electronic shield and thus enabling its rupture [5, 33, 42]. Hence, the addition of aerobic post-treatment or the combination of anaerobic and aerobic environments to mineralize them to CO_2 and water is required [59, 121].

Table 2 Important parameters and efficiencies of aerobic systems used for degradation of azo dyes

Reactor type	Azo dye and concentration	COD (mg/L)	HRT (hours)	Electron donors	Biomass type	Color removal (%)	COD removal (%)	Reference
250 mL shaking flasks	Acid Brilliant Red GR (20–140 mg/L)	2–14 g/L of sucrose	24	Sucrose	–	97	–	[119]
250 mL shaking flasks	Acid Red B (50–300 mg/L)	–	8	–	Suspended growing and immobilized cells	97	–	[118]
Aerated SBB[a]	Acid Red 151 (25–50 mg/L)	–	Each cycle of the SBB consisted of four periods controlled by a timer: fill and draw (3 and 13 min, respectively), reaction (variable) and settle (30 min)	–	Immobilized	73	60	Buitron et al. (2004)
Aerobic granules	Acid Red 14 and Reactive Blue 19 (20–50 mg/L)	–	3.8 (228 min)	–	Aerobic granules	50–80	–	[74]
Aerobic	Indigo dye	–	96 (4 days)	–	Immobilized	97	97	61

[a]SBB = Sequencing Batch Biofilter
[b]WWTP = Wastewater Treatment Plant

The combination of an anaerobic process followed by an aerobic one for the treatment of textile effluents has already been highlighted in the early 1990s. A study by [50] with the azo dye Mordant Yellow (MY) 3, using mixed culture, switched the researchers' focus from treatments with a single anaerobic or aerobic unit to studying the combination of both environments. The authors verified that the complete mineralization of MY3 happened with the alternation between these processes. Thus, studies using aerobic-anaerobic packed bed reactors, aerobic-anaerobic fluidized bed reactors, and aerobic-anaerobic sequential batch or continuous-flow reactors have been better developed, and their systems were improved for the mineralization of azo dyes [9]. Anaerobic-aerobic combination has produced good results in studies conducted with synthetic and real effluents [1, 2, 16, 36, 41, 106].

Nonetheless, this combination results in the use of two treatment units, substantial energy demands, and trained personnel. Furthermore, aromatic amines are produced as intermediate compounds in the anaerobic degradation of azo dyes and are known to have toxicity equal to or greater than that of azo dyes, and many of these amines are carcinogenic [29, 97, 100]. It is noteworthy that the aerobic stage must be reached because it is necessary for the toxicity arising from azo dyes to be reduced.

The aromatic amines produced by the reduction of the azo bond are known to be mutagenic and carcinogenic and may have a higher degree of toxicity than the dye that originated them [13, 17, 21, 57]. Several studies on azo dye reduction indicate that most of the aromatic amines, produced in an anaerobic environment, are capable to be removed in an aerobic environment [1, 2, 16, 97, 121], thus confirming that two-stage systems are an excellent alternative for the complete removal of both the azo dyes and the byproducts generated in the treatment of wastewater containing these compounds.

4 Anaerobic-Aerobic Reactors

Due to the need for sequential anaerobic and aerobic environments for the mineralization of azo dyes, in a process similar to the one shown in Fig. 5, many studies have evaluated the efficiency and optimization of treating systems using a single reactor or sequential reactors under anaerobic and aerobic conditions. The application of these environments can be performed by one- or two-stage systems. In one-stage systems, the anaerobic and aerobic environments occur in the same compartment by alternating anaerobic and aerobic periods or using technologies that provide an oxygen concentration gradient for the microbial community, such as aerobic granules or biofilm reactors. In two-stage systems, firstly an anaerobic reactor is used, where microorganisms perform the reductive cleavage of azo bonds (first stage), followed by an aerobic reactor where the azo dye byproducts formed in the first stage are mineralized by aerobic or facultative organisms. In these systems, the anaerobic and aerobic biomasses are separated by two or more reactors.

Two-stage systems benefit from the separation of anaerobic and aerobic environments to provide specialized microbial communities necessary to perform the

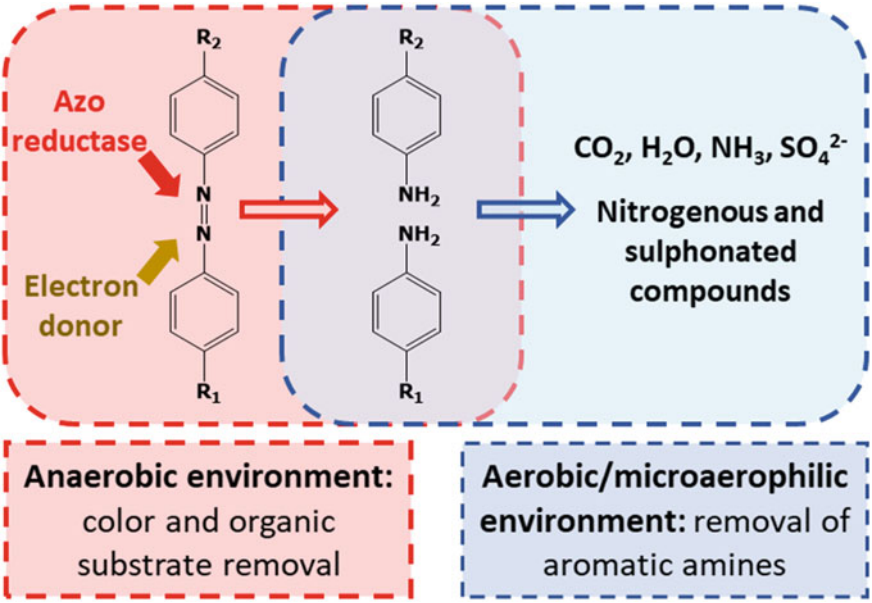

Fig. 5 Mineralization of azo dyes in sequential anaerobic-aerobic process

required reactions at each stage, without the anaerobic organisms being harmed by the presence of oxygen and the aerobic organisms, by the absence of oxygen. Nonetheless, some aromatic amines are not mineralized during the sequential anaerobic-aerobic stages, due to the need for other reactions that only occur in anoxic or microaerated environments [67, 73, 76, 122].

One-stage systems utilize the metabolic synergy of mixed microbial communities (presence of anaerobic, facultative, and aerobic organisms) to perform the metabolic reactions necessary for the mineralization of azo dyes. The variation of redox potential caused by the change between the anaerobic and aerobic environments in the same compartment might favor the degradation of recalcitrant aromatic amines, allowing specific reactions to happen, and consequently, the mineralization of aromatic amines that would not be degraded otherwise, in conventional two-stage anaerobic-aerobic reactors [124]. However, alternating between anaerobic and aerobic environments might harm the development of strict anaerobic or aerobic organisms, reducing the efficiency of one-stage systems [12, 76]. More recently, the possibility of using microaeration (continuous or intermittent) as the aerobic period has been observed [16, 79, 91]. Microaeration can reduce energy costs and minimize the harm to strict anaerobic organisms because of exposure to oxygen.

4.1 Two-Stage Systems

UASB reactors and their variations are widely used in bench and pilot-scale studies as the first stage for the removal of organic matter and decolorization of synthetic and real textile wastewater in sequential two-stage anaerobic-aerobic systems [1, 2, 36, 90, 114, 125]. This reactor is commonly used for its advantage of being compact, of easy operation, and robust, capable of receiving a variety of organic loads while maintaining stable operation even during its variations [23, 109]. Expanded granular sludge bed reactors (EGSB) have also been used for the degradation of azo dyes, presenting a more compact configuration than UASB and allowing intense contact and mixing of biomass and wastewater [30–32]. The parameters that influence the performance of these reactors are the same as seen in the anaerobic reactors.

Different aerobic reactors have been used as the second stage of UASB or EGSB reactors. O'Neill et al. [90] used a bench-scale conventional activated sludge as the second stage of a UASB reactor employed for the degradation of 150 mg/L of Procion Red H-E7B, an azo dye present in a synthetic textile wastewater. Activated sludge is well established and is the most popular system for municipal wastewater treatment. The system uses a completely mixed aerated tank continuously fed, where suspended microorganisms perform the biological treatment, followed by a settler that returns a part of the sludge to the aerated tank. In this study, the UASB reactor, which was operated with HRT of 24 h, was responsible for 60–61% of organic matter removal, while the total system removed from 66 to 88%. In general, it is commonly found that the anaerobic reactor is responsible for most of the organic matter removal. This behavior is favorable for biogas production, which is ideal for industrial applications. The system had maximum color removal of 77% while being fed with azo dye and 3.8 g/L of starch. The authors reported that increasing the ratio between starch and dye improved color removal in the system. In the aerobic reactor, microbial growth was limited by the low availability of carbon sources, since the growth of aerobic organisms has a much faster substrate consumption kinetics compared to anaerobic organisms.

Tan et al. [120] used a combination of EGSB and activated sludge to degrade synthetic wastewater containing the azo dye Mordant Yellow 10 and methanol as the electron donor. The EGSB reactor was operated with HRT varying from 16.8 to 29.3 h, which resulted in color removal higher than 97% even with azo dye concentration ranging from 57 to 192 mg/L. The first stage removed 100% of the methanol used, in all tested conditions. The authors reported minimum degradation of 88% of the aromatic amine 5-aminosalicylic acid in the aerobic reactor, but it was necessary to increase HRT in the aerobic reactor from 8.9 to 79.2 h to promote the development of organisms capable of degrading sulfanilic acid, which is another aromatic amine produced from the reductive cleavage of the azo bonds from Mordant Yellow 10. The system showed great color and organic matter removal; however, in order to degrade the aromatic amines produced, it was necessary to greatly increase the HRT of the aerobic system.

Amaral et al. [2] used a Submerged Aerated Biofilter—SAB as the second stage of a UASB in a pilot-scale system for the degradation of real textile wastewater from a medium-sized industry. Expanded clay spheres were used as a fixed support material inside the SAB. The biofilters allow the growth and maintenance of slow-growing organisms, such as those necessary for aromatic amine mineralization [34]. The authors reported that varying organic load from 1.84 to 2.42 kg of organic matter/m^3.d^{-1} (HRT varying from 8 to 12) in the UASB reactors did not cause significant variation in the efficiency of the system. Nevertheless, the variation of wastewater composition highly influenced the decolorization efficiency of the system. SAB, which was operated with 6 and 9 h of HRT, being an aerobic reactor, was responsible for most of the color removal, with efficiencies ranging from 65 to 92%, while UASB showed 30–52% of color removal. The low color removal performance of UASB was attributed to the high salinity and sulfate concentration of the wastewater, which both inhibited the development of organisms in the anaerobic reactor and caused competition between sulfate and azo dye for the electron donors. The treating system was able to almost completely remove toxicity, as evaluated by ecotoxicity assays using *Daphnia magna* as bioindicator. This study highlighted the robustness of the SAB reactor, using simple support material, for treating real, highly varying textile wastewater.

Sequencing Batch Reactors (SBRs) have also been widely employed in the studies on azo dye degradation; however, they are mostly used as one-stage systems. Therefore, there are few studies that have used SBR in two-stage systems [12, 65, 66]. These reactors are versatile, since they can be manufactured in different volumes, from microcosms to full scale, they can be employed with suspended or granular biomass or with carriers for biofilm growth.

SBR can be used as an anaerobic or aerobic reactor in two-stage systems. Bonakdarpour et al. [12] used two SBR, one anaerobic and one aerobic, for the degradation of a synthetic textile wastewater containing glucose at the concentration of 2 g/L of organic matter and the azo dye Reactive Black 5 at a concentration ranging from 100 to 500 mg/L. SBR was operated at 30 °C for 45 h for each anaerobic and aerobic reaction. In this study, more than 80% of color and organic matter removal were observed in the anaerobic SBR, which is in agreement with most studies. It was reported that increasing azo dye concentration from 100 to 500 mg/L resulted in a slight decrease in anaerobic organic matter removal, indicating a possible toxicity of the azo dye and its metabolites to the anaerobic microbial community. The authors also reported 40–44% of aromatic amine removal in the aerobic SBR and reappearance of color, indicating autoxidation of aromatic amines. In this case, it is possible that the long HRT used in this system was favorable for the first stage, although the long exposure to oxygen might have resulted in the autoxidation of aromatic amines.

Koupaie et al. [66] used Moving Bed Biofilm Reactors (MBBR) as the second stage in a system used to degrade synthetic textile wastewater containing the azo dye Acid Red 18 (AR18) and a mix of glucose and lactose as electron donors. MBBR uses carriers for the development of microbial growth by biofilm formation, similarly to the SAB reactor. The major difference between MBBR and SAB is that in MBBR, the media support moves freely inside the reactor, while it in the SAB, it is fixed. Biofilm

reactors have the advantage of maintaining slow-growing organisms and an oxygen gradient inside the biofilm that will allow the development of diversified organisms. In this study, an anaerobic SBR was used as the first stage, operating with 66 h of HRT and with the temperature controlled at 35 °C. The reactors were fed with wastewater containing glucose and lactose (1.5 g/L of each) and 100–1000 mg/L of AR18. More than 98% of decolorization was reported, and organic matter removal was higher than 80% in the anaerobic SBR. Increasing dye concentration did not cause a significant change in color and organic matter removal efficiency. MBBR operated with 66 h of HRT and was responsible for the total color removal of the system and almost total residual organic matter removal. The authors identified the degradation of 1-naphthylamine-4-sulfonate ranging from 88.7 to 100%, indicating the system efficiency for the degradation of aromatic amines. The concentration of biomass attached to the carriers decreased with the increase in dye concentration, indicating that the metabolites might have inhibited the development of biomass in the MBBR. The combination of SBR-MBBR resulted in excellent azo dye decolorization, organic matter removal, and metabolite removal, while treating a synthetic textile wastewater.

Anaerobic Baffled Reactor (ABR) has also been used in textile wastewater treating systems. ABR is a continuous flux reactor in which multiple compartments are grouped together, sequentially, where the effluent passes through biomasses in each compartment, being gradually treated. ABR compartments turn these reactors into systems of several stages, which results in the development of multiple specialized microbial communities, in addition to being able to avoid long-term contact between toxic compounds and more sensitive organisms, such as methanogenic archaea and the metabolites from azo dye degradation [88, 126, 127].

Zhu et al. [126] used two baffled reactors, each with six identical compartments in which the three initial compartments were anaerobic, followed by two aerobic and one for sedimentation, operating with a total HRT of 24 h. Each compartment had a baffle that made the wastewater follow a downflow and an up-flow while in contact with the biomass. The authors evaluated the degradation of 30–60 mg/L of the azo dyes Acid Orange 7 (AO7) and Methyl Orange (MO) while using 2 g/L of glucose as electron donor. Organic matter was gradually removed along the compartments, without distinct removal efficiency among them, until reaching a total removal of 83.3–83.8%, in the reactors fed with AO7 and MO, respectively. While the aerobic compartments were efficient for aromatic amine removal, the anaerobic compartments also showed the ability to partially degrade aromatic amines. The authors reported dissolved oxygen concentrations higher than 1 mg/L, indicating that they were not effectively hermetic.

A variety of aerobic reactors were successfully used as second stage for mineralization of azo dyes and Fig. 6 shows a didactic scheme of the most used. Table 3 presents the studies that have used different two-stage system configurations for the degradation of azo dyes in synthetic wastewater or real textile wastewater.

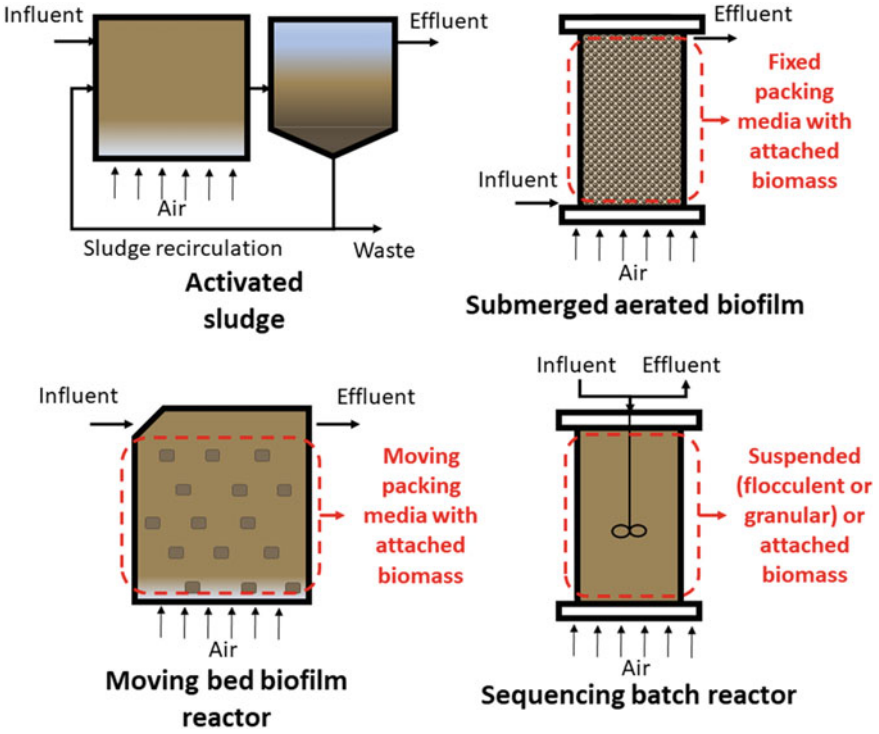

Fig. 6 Schematics of common aerobic reactors used as second stage for degradation of azo dyes by-products

4.2 One-Stage Systems

SBRs were pioneering reactors in the study of azo dye degradation using one-stage systems [75, 76, 120]. In these reactors, the possibility of applying multiple environments along the same reaction cycle (anaerobic, aerobic, microaerophilic or intermittently aerobic/anaerobic) makes them ideal for use in the degradation of azo dyes.

Lourenço et al. [76] studied the degradation of the dye Remazol Brilliant Violet 5R (RBV5R) at concentrations from 60 to 100 mg/L and Remazol Black B (RBB) at the concentration of 30 mg/L, using the starch-derived compound Emsize E1 at a concentration that resulted in 750 mg COD/L. The reactors operated in a 24-h cycle, divided into 21 h of reaction and 2 h for volumetric exchange. The reaction was divided into 9–13 h of anaerobic reaction followed by 8–9 of aerobic reaction. The authors reported the efficiencies of organic matter and color removal of 90 and 80%, respectively, in the reactor fed with RBV5R. The authors also reported that the aromatic amines formed from the anaerobic degradation of RBV5R were partially mineralized during the aerobic reaction, but that increasing aerobic reaction