Water: Analysis, Treatment, and Reuse

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Water quality is an important issue and its degradation is a major threat for everyone. A large quantity of water is wasted as wastewater arising from several activities such as industrial, and agricultural. The environmental pollution arising from the discharge of untreated wastewater is increasing and the quest for finding different water treatment methods is highly encouraged.

In this special issue, we aimed to gather recent researches concerned with water analysis and new developed methods and materials used for water decontamination. Our special issue contains six relevant research articles for authors from Hungary, Zimbabwe, China, Spain, Korea, and India.

The article authored by A. Szekacs et al. is a survey monitoring pesticide residues in surface and ground water in Hungary. In this survey, more than two thousand water samples have been analyzed and the effects of pesticide contamination on ecological farming and drinking water supply have been assessed. In this special issue, F. Chigondo et al. investigated the extraction of alum (a coagulant that is used for raw water treatment) from locally abundant kaolin clays using sulphuric acid. Also, J. Yang et al. reported their successful results for establishing a three-dimensional model integrating computational fluid dynamics (CFD) and biokinetics to model an expanded granular sludge bed reactor. The article by T. Llano et al. describes the evolution of lignin, sugars, and other decomposition products derived from hemicelluloses and cellulose in a sulfite pulp mill. H. Lade et al. reported the use of *Providencia rettgeri* strain HSL1 as a low-cost growth medium for the enrichment of bacteria and their further use for biodegradation of azo dye and its derivatives containing wastes into nontoxic form. And the article authored by Z. Jing et al. described the degradation characteristics of aniline during ozonation.

**Acknowledgments**

Finally, the guest editors wish to express their thanks to all those who have contributed to this special issue, especially the authors and the reviewers of the articles. They thank the editorial staff of the journal for their help in managing the issue.

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Degradation Characteristics of Aniline with Ozonation and Subsequent Treatment Analysis

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1. Introduction

Aniline, the most typical compound in aromatic amines, is a kind of colorless oily liquid with sweet smelling. Aniline is an important raw material and intermediate for organic chemistry [1], which is widely used in some industries such as pesticide [2], medicine [3], oil paint, dyes [4], plastic [5], military, and defense products [6]. Aniline is detrimental to both environment and human's health. It enters the human body by skin, respiratory tract, and digestion system, resulting in carcinogenic, teratogenic, and mutagenic effects on human being [7]. When aniline is discharged into water bodies, it usually disturbs water environment and brings about serious water pollution and even the death of aquatic animals and plants [8]. Because aniline is widely used in many industries, aniline exists in different kinds of industrial wastewater and municipal wastewater. Due to the high toxicity and accumulation of aniline in the environment, more and more rigorous limits on the letting amount of aniline have been established in many countries and districts [9]. When aniline in wastewater exceeds a certain concentration, it will cause detrimental effects to microorganisms in the treatment processes. Consequently, it must be removed or transformed into biodegradable substances before biological treatment processes.

The regular treatment methods for aniline removal include physical treatment, chemical treatment, and biological treatment [10]. In the physical methods, adsorption with activated carbon and macroporous resin is widely used [11, 12]. There are also other methods such as organic solvent extraction and membrane separation. However, the cost of these methods is usually high. In the chemical methods, advanced oxidation processes such as catalytic oxidation [13], ozonation [14], electrochemical degradation method [15], and ultrasonic degradation [16] have been proven effective in aniline removal. Nevertheless, these methods also have the problem of high cost and complicated maintenance [17]. Moreover, most of these advanced oxidation methods can only transform aniline into many intermediates, which also cause adverse effect to water environment. There are also some researches using biological processes as activated sludge system [18], biological contact oxidation, and anaerobic treatment [19]. However, direct biological treatment usually needs long time cultivation for the microorganisms...
to accommodate to the wastewater with high level of harmful aniline [20]. It is also sensitive and fragile to shock load, resulting in unstable performances.

In this study, aniline was disposed by ozonation. Aniline removal and the corresponding COD (Chemical Oxygen Demand) removal were determined during the ozonation. The effects of contact time, initial aniline concentration, ozone dosage, temperature, and pH on aniline and COD removal were studied. The possible degradation routes and intermediates during ozonation were also investigated. The appropriate process with the integrated system of ozonation and biological treatment was suggested.

2. Material and Methods

2.1. Reactor. 1 L of aniline solution with certain initial concentration was put into a graduated glass cylinder. An aerator was connected with an ozone generator and then put at the bottom of the cylinder. The influence of contact time, initial aniline concentration, ozone dosage, temperature, and pH on aniline degradation was investigated. Ozone was produced with an ozone generator (CP-G-3, ozone generation capacity: 2.5 g/h, Qingdao Guolin Industry Co., Ltd., China) with dry air. Ozone dosage was controlled in the range of 10–45 mg/L by calculation from the ozone gas composition changes before and after the ozonation.

2.2. Water Matrix. The aniline solution was made with distilled water with certain dosage of aniline. The pH of the solutions was adjusted with 1 mol/L solutions of HCl or NaOH. Aniline and the corresponding COD concentration during ozonation were measured.

2.3. Analytical Methods. The samples were taken from the cylinder reactor at certain time. The concentrations of aniline and COD were analyzed according to the standard methods [21]. The possible products of ozonation were measured with a GC-MS (Trace DSQ, Thermo Fisher Scientific, Waltham, USA). It used a DB-5S capillary column (30 m × 0.25 mm × 0.25 μm) with helium as the carrier gas at a flow rate of 1 mL/min. The GC column oven temperature was held at 50°C for 3 min and then programmed heating from 50 to 280°C at a rate of 10°C/min, with a final hold time of 5 min. The sampling temperature was controlled at 260°C. The injection was conducted in the splitless mode with injection volume of 1 μL. The mass spectrometer was operated in the electron ionization mode (70 eV) and a source temperature of 250°C. Before GC-MS, the water samples were filtrated with 0.45 μm polyethersulfone membrane and then extracted with n-hexane at a dosage of 5 mL for 25 mL filtrate. The extracted solution was used for GC-MS analysis.

3. Results and Discussion

3.1. The Effect of Contact Time on Aniline Ozonation. The effect of contact time on aniline ozonation was studied at pH 7 and 20°C with ozone dosage of 22 mg/L. Figure 1 showed the variation of aniline and COD removal with time extension during the ozonation.

During the ozonation, the concentration of aniline decreased with contact time extension. In two hours’ ozonation, aniline decreased from 103.81 mg/L to 6.68 mg/L with removal rate of 93.57%, which indicated the excellent degradation effect of ozonation on aniline.

Although the ozonation could remove most of the aniline during the ozonation, the COD removal rate only attained 31.03% in two hours, which indicated most of the aniline was transformed into intermediate products. During the ozonation, aniline solution showed a series of colors as pink, purplish red, reddish orange, orange, yellow, reddish brown, and light yellow. These complicated colors during aniline's ozonation implied that many intermediate products were produced from aniline's transformation. According to the results of GC-MS, the main intermediates during ozonation of aniline were benzoquinone. There were also nitrobenzene and nitroaniline. These products were related with orange, yellow, and brown colors according to their concentration and existing state during the ozonation. Because the azyl on the benzene ring was susceptible to being attacked by ozone and hydroxyl radicals, there were also some intermediates such as benzenediamine, which showed the color of pink and purplish red. The final solution color of light yellow indicated that most of these intermediates were further degraded during ozonation. However, these intermediates could not be completely mineralized by ozonation due to the formation of partial oxidation products relatively unreactive towards ozone [22], which still represented much COD in the solution. If the residual COD was to be removed, other oxidation methods or biological processes would be needed.

3.2. The Effect of Initial Aniline Concentration on Ozonation. At 20°C and ozone dosage of 22 mg/L, the influence of initial aniline concentration was showed in Figure 2. With the initial aniline concentration rise, both aniline and COD removal decreased. At initial aniline concentration of 50 mg/L, aniline and COD removal, respectively, reached 96.59% and 50.00% after two hours’ ozonation. When the initial aniline
concentration was increased to 250 mg/L, the corresponding removal rates of aniline and COD were only 68.28% and 21.44%, respectively.

When the initial aniline concentration was high, the ozonation was overloaded, and the organic compounds could not be transformed into intermediates completely. At the lowest initial aniline concentration, aniline was almost transformed into intermediates, and about half of the intermediates were further degraded into carbon dioxide and water. When most of the aniline was transformed into intermediates, the intermediates could be easily removed by further processes such as biological treatment [23].

3.3. The Effect of Ozone Dosage on Aniline Ozonation. At 20°C, pH 7, the effect of ozone dosage on aniline ozonation was studied with ozone dosage ranging from 10 mg/L to 45 mg/L (Figure 3). The increase of ozone dosage accelerated aniline and COD removal obviously. At ozone dosage of 10 mg/L, aniline removal was 85.94% after two hours’ ozonation. When the dosage was increased to 45 mg/L, 97.19% of aniline was eliminated in two hours, and the removal at 80 min reached 91.94%. These results indicated ozone dosage rise accelerated the aniline's degradation. Most of aniline could be removed under these three levels of ozone dosage from 10 mg/L to 45 mg/L. COD removal at these three levels of ozone dosage after two hours’ ozonation was 19.31%, 31.03%, and 88.28%, respectively. Ozone dosage of 45 mg/L got the highest COD removal, which indicated most of the intermediates could be further degraded into carbon dioxide and water with enough ozone. But this dosage was much higher than the necessary ozone dosage for aniline’s transformation, and there were still some intermediates that could not react with ozone.

In this experiment, there was only aniline in water. If there were other organic compounds coexisting in water, the increase of ozone dosage would increase the production of intermediates. Consequently, it is not economical to COD removal with ozonation alone [24]. The main purpose of ozonation should be to transform the complicated compounds into easily biodegradable intermediates.
3.4. The Effect of Temperature on Aniline Ozonation. The effect of temperature on aniline's ozonation was studied with the temperature changed in the range of 20°C–60°C (Figure 4). It can be seen that the effect of temperature variation was minor on aniline's ozonation. With the temperature rising from 20°C to 60°C, aniline removal only decreased from 92.83% to 88.26%, while the corresponding COD removal decreased from 41.43% to 30.00%. On one aspect, the temperature rise decreased the solubility of ozone in the water and accelerated the escape of ozone from the water [25], which affected the effect of aniline's degradation by ozonation. On another aspect, the temperature rise also speeded up the production of hydroxyl radicals with high oxidation capability [26]. The two effects above happened simultaneously and offset the influence of each other during temperature rise. Consequently, the temperature variation brought out slight effect on aniline's removal, and the ozonation of aniline could be operated at room temperature with high efficiency.

3.5. The Effect of pH on Aniline Ozonation. With ozone dosage of 22 mg/L and temperature at 20°C, the effect of pH on aniline ozonation was studied with pH variation in the range of 3–11 (Figure 5). With pH rise, the removal of aniline and COD increased obviously. Aniline removal increased from 58.61% at pH 3 to 97.00% at pH 11, while COD removal increased from 31.43% to 80.00%. At pH 7, the ozonation removed 88.68% of aniline and 63.57% of COD. These data indicated aniline was susceptible to be degraded in alkaline conditions. This might be caused by the fact that ozone produced more hydroxyl radicals in alkaline conditions, which had a higher oxidation potential and could react more rapidly with most organic compounds compared with ozone molecules [27, 28]. The hydroxyl radicals reacted with the intermediates during aniline ozonation, which accelerated both aniline removal and COD removal.

At different pH conditions, the ozonation of aniline showed many colors' variation. At alkaline conditions, there were drastic foams and offensive odor during aniline ozonation, which indicated the fast degradation of aniline and its intermediates during ozonation. At acid conditions, the colors variation, foams, and odor were not so obvious. At neutral pH, the ozonation got satisfying performance in aniline removal compared with that at alkaline and acid conditions. This is significant for aniline's ozonation under neutral pH with simple operation and low cost.

3.6. The Analysis of Intermediates and Degradation Routes during Aniline's Ozonation. The GC-MS spectrum of aniline wastewater showed peak value at 4.95 min (Figure 6(a)). After two hours' ozonation, the removal of aniline was above 90%. Figure 6(b) showed the peak value at 4.95 min decreased greatly and many other peak values appeared at 7.58 min, 11.57 min, 14.00 min, and so on. During ozonation, the aniline was gradually degraded into organic acids with low molecules such as butane diacid, oxalic acid, and formic acid [29]. The degradation of aniline during ozonation included tree processes: (1) the elementary phase: the main product was benzoquinone; (2) organic acids formation phase: the products of the elementary phase were further degraded into organic acids. At the beginning, the main product was butane diacid. After that, the concentration of oxalic acid increased, indicating the further degradation of organic compounds with long carbon chain; (3) the final degradation phase: the organic acids were degraded into the final products as carbon dioxide and water.

Except the compounds above, the azyl on the benzene ring was susceptible to being attacked by ozone and hydroxyl radicals. Consequently, there were many intermediates with imine groups which were mostly colored products [30].

4. Conclusions

With ozone dosage of 22 mg/L, neutral pH, and room temperature, the ozonation removed aniline efficiently. After two hours' ozonation, aniline removal reached 93.57%, and the corresponding COD could not be removed completely.
After two hours’ ozonation, the corresponding COD removal was only 31.03%.

pH variation affected aniline removal obviously. At alkaline conditions, the aniline was more susceptible to being removed by ozonation owing to more hydroxyl radicals’ production.

The results of GC-MS indicated many intermediates were produced during the process of ozonation such as butane diacid, oxalic acid, and formic acid. In the later phase, the proportion of organic compounds with low molecule increased. There were also many colored intermediates.

The intermediates produced during ozonation were more degradable than aniline; thus the ozonation of such organic compounds as aniline could be integrated with biological processes for further removal.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Evolution of Lignocellulosic Macrocomponents in the Wastewater Streams of a Sulfite Pulp Mill: A Preliminary Biorefining Approach

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1. Introduction

Mixed C5 and C6 hemicellulose sugar platforms serve as feedstock for fermentation producing biofuels such as ethanol or butanol, biopolymers such as polyhydroxybutyrate (PHB), or polybutylene succinate (PBS), and chemicals such as lactic acid, succinic acid, or itaconic or glutamic acids [1]. In Europe, primary energy consumption is dominated by the presence of petroleum products mostly imported from abroad. There are many concerns around the high degree of energy dependence [2]. Among the valorization alternatives described above, this work is based on bioethanol production from the pulp and paper industry, in order to be included in the transportation sector.

The pulp and paper industry is being reconsidered as an important source of hemicellulose carbohydrates. Traditionally, pulping manufacturing was focused on cellulose extraction by chemical, mechanical or semichemical processes. Among the chemical processes, kraft is the most commonly used. However, other processes such as soda-anthraquinone (soda-AQ); ethanol-water pulping (organosolv); acid sulfite and bisulfite pulping; sulfite pretreatment to overcome recalcitrance of lignocellulosic (SPORL); or SO2-ethanol-water process (SEW) can also be used [3–10]. Sulfite pulping is becoming popular because of the growing demand of high purity dissolving pulp for textile fiber production. An advantage of sulfite pulping is regarding high separation efficiency of cellulose. While dissolving cellulose is manufactured, hemicellulose and lignin are also generated and partially reused. Conversion of these waste streams is becoming a clear priority within the biorefinery concept.

Nowadays, there are many efforts from the pulp and paper industries focused on sugar-rich resource valorization into energy and a wide variety of products. Several approaches
tracing pathways and guidelines towards the conversion of pulping factories into lignocellulosic biorefineries (LCBR) can be found in the literature for kraft pulping [11–14], soda-AQ [15, 16], organosolv [17–19], or SEW process [9, 10]. Nevertheless, only a few contributions have been studied in the case of sulfite pulping [20, 21]. The investment activity in this field reveals the importance of transforming traditional pulping factories into integrated LCBR, increasing the profit margin in the existing pulp mills. Due to the complexity of the lignocellulosic biomass (LCB), many efforts are being carried out in fractionation processes. In this sense, the first step was a deep control of the main resources throughout a total mass balance of the LCB. The mass balance provides a complete description based on lignin and carbohydrate content (hemicellulose and cellulose) of the materials [9, 22]. The use of total mass balances in a whole process can point out the main resources for valorization options. In addition, the study of digestion and bleaching allows the establishment of future actions towards process improvements. One of the methodologies to get the total mass balance in these kinds of samples is the summative analysis of the carbohydrates disclosure into their derivatives together with lignin, extractives, and ash contribution [22–24]. One advantage of this methodology is the possibility of the delignification and breakdown of the polysaccharides into individual sugars and other decomposition products, giving some information about possible inhibitors in future fermentation processes. This methodology was previously reported with purposes related to the characterization of bagasse and bamboo [23], different wood species [22–27], or pulps [28–32]. More recently, spent liquors provided from SEW were also characterized by summative analysis methodologies, tracing mass balances for the residue bioconversion towards butanol, ethanol, and acetone/isopropanol [9, 10].

This work contemplates the study of the summative analysis in a sulfite pulp mill, monitoring the three main wood macrocomponents—cellulose, hemicellulose, and lignin—by measuring not only the raw material but also the residues and products throughout the process. In a second step, bioethanol potentials of the sugar-rich residue were also determined. This research constitutes a novelty in an industrial sulfite process towards the conversion of this factory into a modern LCBR. In addition, thanks to the compositional analysis together with the physic-chemical pulp properties (viscosity and micro kappa were also measured) the effects of digestion and bleaching steps were also investigated. The proposed methodology can be usefully extrapolated in not only sulfite or kraft mills but also other factories working with LCB.

2. Materials and Methods

2.1. Materials and Industrial Process Description. Figure 1 shows the industrial process and all of the materials collected in the pulp mill. The acid sulfite process is based on the extraction of cellulose by the attack of an acidic aqueous solution under acidic conditions (pH of 1.35 ± 0.15) in the presence of excess free $\text{SO}_2$ [2]. Delignification occurs inside the digester (see “D” unit in Figure 1) where the lignin is sulfonated by $\text{HSO}_4^-$ forming lignosulfonates. Lignosulfonates together with high amounts of depolymerized hemicelluloses are dissolved into the so-called spent sulfite liquor (SSL) obtained at the end of the digestion stage. After digestion, the next step is bleaching (see “Z/EOP/PO” bleaching sequence in Figure 1). With the purpose of purifying the cellulose,
a total chlorine free (TCF) bleaching process is used in the factory. Ozone (Z), sodium hydroxide as the extracting agent together with oxygen and hydrogen peroxide (EOP), and hydrogen peroxide as the bleaching agent in the presence of oxygen (PO) are the stages followed in the pulp mill and studied in this work.

Wood, SSL, and pulp industrial samples were collected throughout the process as can be seen in Figure 1. *Eucalyptus globulus* timber is used as feedstock in this factory. On the other hand, SSL and industrial dissolving pulp samples were collected and analyzed in the process. A total of four delignification-grade pulps were analyzed starting with the crude pulp (P1) after the digestion stage and continuing with the bleaching stages after ozonation (P2); alkaline-extraction (P3); and peroxideoxygen bleaching (P4).

### 2.2. Analysis of Wood, Pulp and SSL

A summary of the characterization methods applied in this work is shown in Table 1. Wood and pulp samples were conditioned and prepared according to TAPPI T257 cm-02 [33]. Samples were air-dried to constant moisture to the nearest 10% w/w, milled, passed through 40-mesh sieve, and extracted with acetone in a Soxhlet apparatus in order to remove the extractives, according to T204 cm-97 standard [33]. Wood and pulp free-extractive samples were taken for the rest of the analysis. Ashes at 525°C were analyzed by TAPPI T211om-02 standard [33]. Acid-insoluble and soluble lignin were determined in wood samples by using TAPPI T222 om-02 method [33]. Cellulose content was determined using the Seifert procedure boiling a mixture of acetylacetone-dioxane-hydrochloric acid [34]. Holocellulose, which represents the total carbohydrate content (as sum of cellulose and hemicelluloses), was measured by means of the Wise chlorite technique reported recently by Haykiri-Acma et al. [35]. The lignin content (% on weight) in pulp was calculated by multiplying the kappa number by 0.17 [32]. Considering that the standard kappa number determination cannot be applied to pulp with kappa below five [41], micro kappa described in TAPPI UM 246 standard [36] was determined. To study the degradation of carbohydrate chains during the bleaching steps, intrinsic viscosity in crude, partially bleached, and bleached pulps was determined by means of the standard ISO 5351:2010 [37]. Alfa-cellulose was also measured in pulp samples using TAPPI standard T203 cm-99 [33].

SSL samples were studied in terms of lignosulfonates, sugars, and other decomposition products. The carbohydrate composition of wood, pulp, and SSL was conducted using HPLC/RID with the methodology published by Llano et al. [38]. Lignosulfonates were analyzed by UV-Vis spectroscopy, according to the UNE EN 16109 standard [39].

### 2.3. Hydrolysis Procedure, Summative Analysis Calculations, and Biofuels Potentials

Polymeric sugars contained in the cell wall of wood carbohydrates need to be broken down. The \(\beta(1 \rightarrow 4)\) glycosidic linkages of the wood polymers are cleaved with the acid hydrolysis method described in TAPPI T249 cm-00 [33]. This method involves a two-step acid hydrolysis: (i) primary hydrolysis uses a strong acid at low temperature to convert the polysaccharides to oligomers, (ii) followed by the dilution to a weak acid at high temperatures to complete the conversion to monomeric sugars. First, free-extractive moisture-controlled samples were weighed at 0.35 ± 0.01 g into flask tubes. Then, 3 mL of 72% w/w H\(_2\)SO\(_4\) was added into the glass test tubes, occasionally stirred in a vortex and maintained 1 h at 30°C into a thermostatic bath. The secondary hydrolysis was carried out at 120°C for 1 h after dilution to 4% by transferring hydrolysates to Duran bottles and adding 84 mL of deionized water (Duran bottles must be hermetically closed). Afterwards, samples were cooled and a representative aliquot of 10 mL was transferred to a beaker.
Several drops of bromophenol blue indicator were taken and gradually neutralized adding 0.04 N Ba(OH)$_2$ alkaline solution to the aliquot until the solution changes from yellow to blue-violet. Then samples were centrifuged and 0.22 μm filtered and injected in the HPLC.

Each monomer can be reported in the summative analysis as its pure theoretical homopolymer [24]. The weight of each constituent, determined quantitatively after the hydrolysis, has to be multiplied by a factor to calculate its contribution to the original wood component (as a theoretical homopolymer). Calculations were made by using the theoretical stoichiometric factors obtained in the literature [22–24]. These factors consist of molecular mass of anhydrous unit divided by molecular mass of the isolated substance. Table 2 shows all of the conversion factors used in this work. Each homopolymer was calculated considering not only the monosaccharides but also the degraded-compounds derived from carbohydrates; for example, cellulose is the sum of cellobiose, glucose, HMF (5-hydroxymethyl-2-furfuraldehyde), and levulinic acid multiplied by their stoichiometric factors. The individual contribution of carbohydrate-derived compounds to the final cellulose or hemicellulose content depends on the chemical structure of the macromolecules forming the cell wall. In this work, all the glucose is assumed to generate from the cellulose [24]. Simultaneously, it was also assumed that formic acid is an inhibitor mostly produced from pentose sugars, being the formation of formic from hexoses negligible compared to the levulinic acid formation [42]. Acetic acid was considered a coproduct formed at the same time as monosaccharides by degradation of the acetyl groups located on the hemicellulose [43].

The macrocomponent calculations from their homopolymers are given by (1). Finally the total mass closure is calculated according to (1). In addition, ethanol potentials were calculated multiplying grams of each monomer by their corresponding stoichiometric factors (see Table 2). Such factors describe the mass fraction of sugar monomers converted to ethanol [40]:

\[
\text{Total Carbohydrate Content} = \text{Cellulose} + \text{Hemicellulose},
\]

\[
\text{Hemicellulose} = \text{Xylan} + \text{Arabinan} + \text{Galactan} + \text{Mannan} + \text{Acetyl},
\]

\[
\text{Cellulose} = \text{Glucan},
\]

\[
\text{Total Mass Closure} = \text{Lignin} + \text{Total Carbohydrates} + \text{Extractives} + \text{Ash}.
\]

### 3. Results

#### 3.1. Total Composition of the Lignocellulosic Samples

The results of the total content per sample are shown in Table 3, including the major components, ash, and extractives. The results represent the total weight percentage content of the industrial samples collected in the pulp mill. The total mass closure was near 100% in spite of the fact that some minority compounds were not analyzed such as low molecular phenolic compounds derived from lignin or aldonic and uronic acids derived from cellulose and hemicellulose.

The comparison of traditional characterization using gravimetric and titration methods and the carbohydrate analysis derived from the summative analysis calculations is displayed in Table 3. Traditional cellulose methods include alfa-cellulose in pulp [33] and Seifert for cellulose in wood [34]. Traditional hemicellulose in wood is calculated as the difference between holocellulose [35] and Seifert cellulose [34].
Cellulose-HPLC and hemicellulose-HPLC of wood and pulp samples were obtained stoichiometrically, after acid hydrolysis of carbohydrates and HPLC sugars quantification. Otherwise, SSL sugars were measured directly in the HPLC, avoiding the hydrolysis step.

Cellulose obtained by traditional methods and cellulose-HPLC in *Eucalyptus globulus* samples present values of 42.25% and 46%, respectively. The Seifert method entails higher experimental errors because of the wood digestion at high temperatures where some projections can be formed if the analysis is not carried out carefully. In pulp samples, the cellulose showed higher values by means of the traditional method. Alfa-cellulose corresponds to the insoluble fraction cellulose showed higher values by means of the traditional method. Alfa-cellulose corresponds to the insoluble fraction cellulose with a lower degree of polymerization are excluded, but considering the results of Table 3, there are chains with similar molecular weights that are also being quantified. Regarding the results of hemicellulose, the hemicellulose-HPLC is lower than hemicellulose calculated by traditional methods in wood 24.92% and 31.55%, respectively. This behavior could be explained by the assumption that the glucose content is only considered to form part of the cellulose fraction. In addition, gravimetric mistakes of Seifert and holocellulose methods are overlapping, giving more errors in comparison with the chromatographic method. An alternative to the study of hemicelluloses in pulp samples can be the pentosan determination with the T223 cm-01 procedure [33]; however, pentosan analysis was not performed in this work because it only contemplates the C5 sugars.

The results of the total carbohydrate content (TCC) disclosure appear in Table 4. TCC of 67.18% and 26.98% of lignin was obtained in *Eucalyptus globulus* hardwood samples. Besides, the replicates checked showed average values of 42.25% cellulose and 77.53% holocellulose. Results of lignin varying from 23% to 27% and cellulose from 45 to 54% of *Eucalyptus globulus* timber were found in the literature [26, 27]. Such ranges are in accordance with the results obtained in this work. The total content of xylan was 13.27% in wood samples, representing more than 50% of the total hemicellulose content. This is because hardwood, in contrast to coniferous softwood with a higher portion of hexosans than pentosans, is composed mainly of pentoses where xylose is the major monosaccharide [11, 44–46]. TCC is much higher in pulp samples in comparison with the *Eucalyptus globulus* samples.

The difference is explained because little amounts of lignin and hemicellulose were found in pulp samples. Hemicellulose decreases from 6.2% to 2.1% and lignin from 0.8% to 0.1% (see Table 3). TCC in pulp samples decreases in the bleaching processes, as can be seen in Table 4, from values of 95.2% to values of 91.8–93.4%. This phenomenon can be explained by the fact that xylan drops from 5.3% to 1.5% despite the fact that cellulose increases from 89.0% to 91.3%.

Once the total carbohydrates were obtained, a theoretical quantity of bioethanol was calculated according to the stoichiometric factors explained in Section 2.3. Results from 0.215 to 0.684 L ethanol per kg of dry sample were obtained.

### Table 3: Total weight content of industrial samples.

| Total mass closure | *E. globulus* (% w/w) | SSL (% w/w) | P1 (% w/w) | P2 (% w/w) | P3 (% w/w) | P4 (% w/w) |
|--------------------|------------------------|-------------|------------|------------|------------|------------|
| Cellulose-HPLC     | 42.25                  | 5.67        | 89         | 87.3       | 89.9       | 91.3       |
| Cellulose          | 46.00                  | —           | 91.34      | 91.16      | 92.36      | 92.28      |
| Hemicellulose-HPLC | 24.92                  | 30.42       | 6.2        | 5.1        | 2.2        | 2.1        |
| Hemicellulose      | 31.55                  | —           | —          | —          | —          | —          |
| Lignin             | 26.98                  | 42.99*      | 0.80       | 0.40       | 0.40       | 0.10       |
| Ash                | 0.35                   | 12.1        | 0.28       | 0.26       | 0.24       | 0.18       |
| Extractives        | 1.5                    | —           | 0.30       | 0.20       | 0.20       | 0.20       |
| TOTAL              | 96                     | 91.18       | 96.58      | 93.26      | 92.94      | 93.88      |

* Lignin in SSL is represented by the lignosulfonate content, formed by lignin sulfonation.

3.2 Dissolving Pulp Properties: Results and Discussion. Pulp properties and their evolution within the sulfite process are represented in Figures 2(a), 2(b), 2(c), and 2(d). Pulp transformation from crude pulp after digestion stage (P1) to final bleached pulp (P4) is graphed with error bars.

Pulp quality parameters are represented in Figures 2(a) and 2(d). Glucan and alfa-cellulose have similar trends, especially in the alkaline extraction process; however some differences can be found in the case of ozonation with a more noticeable decrease of glucan. Pulp impurities were plotted, respectively, in Figures 2(b) and 2(c). In this case, similar results were obtained. Lignin and hemicellulose content decreases as the process advances. These results showed that the most oxidative stage is the ozonation where the main losses of lignin are registered from 0.8% to 0.4%. Although delignification is the main function of this stage, there is also a depolymerization of hemicelluloses from 6.2% to 5.1% because of the high oxidation produced by ozone. In spite of the recalcitrant nature of cellulose with no losses of alfa-cellulose, there is also a little decrease of glucan from 88.8% up to 87.3% probably due to the degradation of beta and gamma cellulose. Such behavior is also reflected in the viscosity falling from 706.4 mL/g to 568.2 mL/g. Figure 2(d) shows the polymerization degree of cellulose chains playing an important role in the quality of the final pulp. As was expected, the viscosity diminished stage by stage from 706.4 mL/g (P1) after digestion up to 492.5 mL/g (P4) after PO bleaching.

The obtained results were compared with other quality pulps as a function of the process (chemical or thermomechanical), the feedstock (softwood or hardwood), and the
Table 4: Total carbohydrate content of the woody hydrolyzates.

|                | Wood SSL | Sulfite dissolving pulps (% w/w) | P1 | P2 | P3 | P4 |
|----------------|----------|----------------------------------|----|----|----|----|
| GLUCAN         | 42.25    | 5.67                             | 89.0|87.3|89.9|91.3|
| Glucose        | 44.99 ± 2.13 | 4.12 ± 1.48                     | 95.0 ± 3.81| 93.7 ± 3.27| 96.7 ± 2.42| 98.1 ± 2.52|
| HMF            | 0.1 ± 0.03  | 0.02 ± 0.009                     | 0.3 ± 0.001| 0.3 ± 0.05| 0.3 ± 0.002| 0.3 ± 0.00|
| Levulinic acid | 0.14 ± 0.03 | 0.01 ± 0.009                     | 0.2 ± 0.09| 0.2 ± 0.01| 0.2 ± 0.02| 0.4 ± 0.00|
| Cellobioso     | 1.51 ± 1.29 | 2.04 ± 0.16                     | 3.1 ± 1.78| 2.5 ± 1.95| 2.4 ± 1.26| 2.2 ± 1.84|
| XYLANS         | 13.27     | 19.15                            | 5.3 |4.5 |1.7 |1.5 |
| Xylose         | 14.27 ± 0.47 | 21.43 ± 8.80                    | 2.9 ± 0.67| 2.7 ± 0.66| 2.0 ± 0.42| 1.7 ± 0.75|
| Furfural       | 0.2 ± 0.19  | 0.15 ± 0.055                     | 0.4 ± 0.004| 0.8 ± 0.02| 0.1 ± 0.003| 0.2 ± 0.001|
| Formic         | 0.15 ± 0.05 | 0.03 ± 0.028                     | 0.3 ± 0.18| ND | ND | ND |
| ARABINAN       | 0.52      | 2.46                             | 0.3 |0.3 |0.2 |0.2 |
| Arabinose      | 0.59 ± 0.36 | 2.79 ± 1.71                     | 0.3 ± 0.13| 0.4 ± 0.16| 0.2 ± 0.09| 0.3 ± 0.16|
| GALACTAN       | 7.36      | 3.03                             | 0.4 |0.3 |0.3 |0.4 |
| Galactose      | 8.18 ± 1.53 | 3.36 ± 1.52                     | 0.4 ± 0.13| 0.4 ± 0.16| 0.3 ± 0.09| 0.4 ± 0.26|
| MANNAN         | 1.00      | 1.28                             | ND | ND | ND | ND |
| Mannose        | 0.01      | 1.42 ± 0.50                      | ND | ND | ND | ND |
| ACETYL         | 2.78      | 4.51                             | 0.2 | ND | ND | ND |
| Acetic         | 3.87 ± 0.32 | 6.30 ± 1.70                     | 0.3 ± 0.24| ND | ND | ND |
| TCC (%)        | 67.18     | 36.09                            | 95.2 |92.4 |91.8 |93.4 |

*EtoH (L/Kg,dw) calculated from the homopolymers using hydrolysis and fermentation factors.
*EtoH (L/Kg,dry sample) calculated from the monomers using fermentation factors.

Figure 2: Evolution of wood macrocomponents in pulp along the sulfite mill: (a) glucan and alfa-cellulose; (b) hemicellulose and xylan; (c) kappa index and lignin; (d) viscosity in pulp.
final application (paper-grade or dissolving grade) [11, 28, 29, 47]. Results presenting major impurity removal are the more suitable for waste streams valorization towards biofuels and other value-added products. The worst pulp quality is the thermomechanical (TMP) pulp with a total carbohydrate content of 64.4% in comparison with chemical pulping processes with a total carbohydrate of 96.5% [30]. The TMP constituted low-purity (regarding the lignin content) and high-yield pulp. The difference between paper-grade and dissolving-grade pulp resides in the total glucan content that is lower in case of paper grade, obtaining values of 74.7% and 84.9% for hardwood and softwood bleached pulps [47] and 92.6% in the case of dissolving-grade pulps [29]. Consequently, the hemicellulose content is higher in paper-grade pulps than in high purity dissolving-grade pulps. In this work, total carbohydrate content in bleached pulp (P4) is 93.4% where 91.3% belongs to glucan with only 1.5% of xylan.

Based on the experimental results shown in Figure 2 it can be concluded that (i) ozonation stage (Z) produces the destruction of mainly lignin and also carbohydrates. Z focuses on delignification and therefore kappa is notably reduced. Nevertheless, glucan is considerably diminished during Z whereas this does not affect the alfa-cellulose. This is due to the fact that the ozone is very aggressive as a bleaching agent (being less selective than chlorine derivatives) and attacks beta and gamma cellulose chains; (ii) hot alkaline extraction stage (EOP) focuses on hemicellulose solubilization, falling hemicel luloses from 5.1% to 2.2% and specifically xylan from 4.5% to 1.7%; (iii) peroxide bleaching stage (PO) attacks the chromophore groups and the pulp is definitely purified by removing lignin traces from 0.4% to 0.1% and other groups responsible for the color of the pulp; (iv) selectivity in PO and Z stages should be improved in order to avoid the breakdown of the cellulose chains; (v) results evidenced the importance of the wastewater streams valorization considering the high charge of organic compounds removed from the high-purity dissolving pulp along the sulfite process.

3.3. Mass Balance of the Industrial Process. The mass balance of the entire industrial process has been carried out taking into account the summative analysis. The complete characterization of the feedstock (Eucalyptus globulus timber), the inlet-outlet pulps, and the main residual stream (SSL) was required. Data of the three macrocomponents throughout the process, flow rates, digestions per day, wood moisture, or yields were considered. Some of the data are confidential to the factory and cannot be specifically displayed. Results appearing in Figure 3 have been correlated with the initial dry wood.
wood in terms of grams of cellulose, hemicellulose, and lignin per grams of dry wood. The main discussion is described as follows:

(i) A total content of 99.6% of cellulose provided from the feedstock goes to the main product, dissolving pulp, indicating the good performance of the digestion process. Only traces of wood cellulose are dissolved into the spent liquor. Thus, 0.032 g.hemicellulose/g.dry wood and 4.1 - 10^-3 g.lignin/g.dry wood were detected in the crude pulp (P1) which will be removed throughout subsequent stages.

(ii) Based on the global mass balance and the conclusions of Section 3.2, some action lines can be made regarding Z and PO stages. A better use of the bleaching reagents and process conditions should be made in order to decrease the depolymerization degree but not to the detriment of delignification.

(iii) The SSL generated after wood digestion is composed of 87.2% of the total hemicellulose in wood (0.218 g.H/g.dw) and 98.5% of the total lignin (0.266 g.L/g.dw). Hemicelluloses are hydrolyzed and dissolved as monosaccharides and other derivatives. Likewise, lignin reacts with sulfite, bisulfite ions, and sulfurous acid forming lignosulfonates. Based on these results SSL can be a perfect candidate for second-generation biofuel production.

The SSL is evaporated in the factory in order to reduce the water content. However, samples collected in this work were collected before the evaporation plant, at the tank outlet (see WSSL in Figure 3). Tap water is used at the end of the digestion stage to stop the hydrolysis and depolymerization reactions. In addition, wastewater streams provided from pulp washing containing cellulose, hemicellulose, and lignin are stored in the tank together with the SSL and sent to the evaporation plant as WSSL. Theoretical bioethanol potential of the WSSL was calculated based on the carbohydrates content (0.031 g.C/g.dw and 0.205 g.H/g.dw). The hydrolysis stoichiometric factors for hexoses and pentoses were, respectively, 1.11 g.C6-sugars/g.cellulose and 1.136 g.C5-sugars/g.hemicellulose; the fermentation stoichiometric factor for ethanol production is 0.511 g. EtOH/g.monosaccharide. Assuming the complete conversion of C5 and C6 sugars, the second-generation bioethanol potential of the WSSL is 0.173 L.EtOH/g.dw.

4. Conclusions

A full study of total mass balance throughout the entire sulfite pulping process in a pulp mill has been carried out, showing that the spent sulfite liquor is the most useful stream to be valorized due to the presence of lignosulfonates, sugars, and other minor compounds, giving a theoretical quantity of 0.173 L of bioethanol per gram of dry wood. Fractionation processes might be carried out to separate the value-added compounds, transforming this traditional pulp mill into a modern lignocellulosic biorefinery.

The characterization of the woody materials has been developed, comparing traditional methods with more novel methods based on the hydrolysis and individual characterization of the monomers. Acid hydrolysis is a useful method for the analysis of carbohydrate composition of wood and pulp samples. Using the TAPPI T249 cm-00 standard in combination with HPLC-RID technique can give complete information of the main components for valorization options in pulping processes.

Summative analysis results together with other parameters make studying every stage of the sulfite process possible. A favorable extraction of cellulose in the digester was carried out, with the presence of 99.6% of wood-cellulose in the crude pulp. On the other hand, 87.23% of hemicellulose and 98.47% of lignin are dissolved into the spent liquor.

Finally, some action lines to the existing process were indicated: (i) the digestion conditions should be optimized in order to increase the depolymerization of hemicelluloses in the spent liquor; (ii) ozonation and peroxide bleaching extraction processes should also be improved, avoiding the degradation and destruction of the cellulose chains and obtaining similar values of impurities; (iii) the spent liquor should be conveniently fractionated and detoxified, separating sugars from the rest of microbial inhibitors for second-generation biofuel production by microbial fermentation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Low-Cost Biodegradation and Detoxification of Textile Azo Dye C.I. Reactive Blue 172 by Providencia rettgeri Strain HSL1

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Present study focuses on exploitation of agricultural waste wheat bran (WB) as growth medium for degradation of textile azo dye C.I. Reactive Blue 172 (RB172) using a single bacterium P. rettgeri strain HSL1 (GenBank accession number JX853768.1). The bacterium was found to completely decolorize 50 mg L⁻¹ of dye RB172 within 20 h at 30 ± 0.2°C under microaerophilic incubation conditions. Additionally, significant reduction in COD (85%) and TOC (52%) contents of dye decolorized medium was observed which suggested its mineralization. Induction in the activities of azoreductase (159%) and NADH-DCIP reductase (88%) provided an evidence for reductive cleavage of dye RB172. The HPLC, FTIR, and GC-MS analysis of decolorized products confirmed the degradation of dye into various metabolites. The proposed metabolic pathway for biodegradation of RB172 has been elucidated which showed the formation of 2 intermediate metabolites, namely, 4-(ethenylsulfonyl) aniline and 1-amino-1-(4-aminophenyl) propan-2-one. The acute and phytotoxicity evaluation of degraded metabolites suggests that bacterial strain favors the detoxification of dye RB172. Thus, WB could be utilized as a low-cost growth medium for the enrichment of bacteria and their further use for biodegradation of azo dyes and its derivatives containing wastes into nontoxic form.

1. Introduction

Synthetic textile dyes are of complex aromatic structures specially designed for chemical stability and versatility and to resist the effect of high temperature during wet processing operations which makes them highly recalcitrant [1]. Thousands of such synthetic dyes are extensively used in the textile industry for dyeing and printing purposes [2]. Approximately, 40–65 L of textile wastewater is produced per kg of cloth during dyeing processes [3]. Among all textile dyestuff used, the azo dyes constitute about 70% and are being used worldwide [4]. The discharge of azo dyes containing wastewaters into the environment may lead to the bioaccumulation which causes toxic effect on aquatic life and even carcinogenic and mutagenic effect on humans because of the conversion of azo group into aromatic amines [5, 6]. Aside from the human toxicity, colour of dyes interrupts the aquatic environment by reducing light penetration, gas solubility, and interference of phytoplankton’s photosynthesis [7]. Therefore, treatment of textile wastewater becomes essential before discharging into the water streams. Additionally, limited supply and increasing cost of water for industrial sector have made the treatment and reuse of dyeing effluent mandatory to avoid the environmental pollution as well as reduce the production cost.

Several physicochemical methodologies such as coagulation and flocculation are most commonly used worldwide for treatment of textile effluent [8, 9]. But some shortcomings such as excessive use of chemicals, secondary pollution, large amount of sludge generation, low efficacies, and high operational cost discourage the employment of these methods [10]. Alternatively, the modern method bioremediation, which utilizes the ability of bacteria, fungi, or its combination system, has emerged as an effective method for the treatment of textile wastewaters [11–13]. However, higher price of microbial growth medium makes biological treatments expensive and beyond the use at commercial levels. Thus, to overcome the problem of higher cost of microbial growth medium and
make the bioremediation an efficient treatment technology, the use of agricultural waste as growth medium has been suggested.

A number of agricultural wastes and its by-products such as sugarcane bagasse, wheat straw, corn cob, rice bran, and wheat bran are cheapest and abundantly available carbon sources [14]. These are normally utilized as animal fodder and domestic fuel while a large portion is being disposed of as waste [15]. For instance, approximately 145.20 million tons per year of wheat straw is available in Asia [16]. However, only a small portion of wheat residues is used as animal feed and the rest is removed from the field by burning which causes air pollution and affects human health [17]. Recently, agricultural waste wheat bran has been used as growth medium for microbial consortium and their further use in biodegradation of azo dye Trypan Blue under submerged conditions [18].

In this view, the easily available agricultural waste wheat bran was further evaluated as a low-cost growth medium for degradation of model azo dye RB 172 using a single culture of P. rettgeri strain HSL1 bacterium under submerged conditions. Initially, the optimization of conditions for enhanced dye degradation efficacy was performed. The activities of dye degrading enzymes laccase, azoreductase, and NADH:DCIP reductase were assayed spectrophotometrically. Mineralization of dye was determined by the reduction in COD and TOC values whereas biodegradation was confirmed by HPLC, FTIR, and GC-MS analysis. Possible metabolic pathway for degradation of dye RB 172 has been constructed. Finally, the environmental risk assessment was performed by acute and phytotoxicity tests.

2. Materials and Methods

2.1. Chemicals and Textile Azo Dye RB 172. 2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), methyl red, nicotinamide adenine dinucleotide (NADH), and dichlorophenolindophenol (DCIP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Textile azo dye RB 172 (CAS number 85782-76-9; molecular formula = C_{28}H_{32}N_{2}O_{10}S_{2}; molecular weight = 702.74) was generously given by Mahesh Textile Processors (Ichalkaranji, MS, India).

2.2. Preparation of WB Medium. Agricultural waste wheat bran was obtained from local market (Kolhapur, MS, India), sieved, and oven-dried at 80°C ± 1.0°C for several hours until the weight was constant, and then 5 gm dry wheat bran was taken in 100 mL distilled water. This content was boiled for 15 min and the extract was separated by filtration through Whatman grade number 1 filter paper which then diluted to 100 mL with distilled water and called AWWB medium. The pH of AWWB medium was adjusted to 7.0, autoclaved for 15 min at 121°C, and used for further degradation experiments.

2.3. Preenrichment of P. rettgeri Strain HSL1. Prior to decolorization experiments the preenrichment of P. rettgeri strain HSL1 was routinely carried out in AWWB medium. A loopful of bacterial stock culture was inoculated in 250 mL Erlenmeyer flask containing 100 mL of AWWB medium (pH 7.0) and incubated at 30 ± 0.2°C for 24 h under shaking conditions (120 rpm). The overnight grown culture was then used as inoculum for further dye decolorization experiments.

2.4. Optimization of Decolorization Conditions. All the decolorization experiments were carried out in 250 mL Erlenmeyer flask containing 100 mL of preenriched P. rettgeri strain HSL1 culture. The optimization of conditions for enhanced decolorization of dye RB 172 was carried out by one parameter approach at a time. Initially, the effect of microaerophilic and aerobic incubation (shaking at 120 rpm), preenriched culture medium pH (3–12), incubation temperature (20, 30, 37, 40, and 50 ± 0.2°C), and dye concentrations (50–250 mg L⁻¹) was evaluated. At defined time of intervals the aliquots of culture supernatant (3 mL) were withdrawn and suspended particles were removed by adding equal volume of methanol followed by centrifugation (7500 x g for 15 min, 4 ± 0.2°C) [13]. The resulted clear supernatant was analyzed for decolorization at maximum absorbance wavelength of 570 nm using UV-vis spectrophotometer (Hitachi U-2800; Hitachi, Tokyo, Japan). The control flasks which were without dye or bacterial culture were also tested under the same conditions. All the experiments were conducted at least in triplicate at 30 ± 0.2°C and average values were calculated. The decolorization was expressed in terms of percent using the formula:

\[
\text{Decolorization (%) } = \frac{\text{Initial absorbance}_{(0h)} - \text{Observed absorbance after incubation}_{(t)}}{\text{Initial absorbance}_{(0h)}} \times 100. \tag{1}
\]

2.5. Dye Mineralization Analysis. The mineralization of dye RB 172 was confirmed by chemical oxygen demand (COD) and total organic carbon (TOC) analysis. For this, the control and decolorized culture broth were centrifuged (7500 x g for 15 min, 4 ± 0.2°C) and filtered through 0.45 μm cellulose acetate filter (Sterlitech Corporation, Kent, WA, USA) to remove cell biomass. The reduction in COD was determined by dichromate closed reflux titrimetric method [19] and TOC by using a Sievers 5310C automated analyzer (GE Water & Process Technologies, Boulder, CO, USA).

2.6. Analysis of Metabolites after RB 172 Decolorization. The extraction of metabolites produced after degradation of RB 172 by P. rettgeri strain HSL1 was carried out by centrifugation (10,000 x g for 20 min, 4 ± 0.2°C). The resulting supernatant was added into an equal volume of ethyl acetate and mixed vigorously to dissolve metabolites. The organic layer was separated, air-evaporated, and dried over anhydrous Na_2SO_4. The remaining metabolite residues were scrapped and dissolved in 3 mL of HPLC grade methanol. Finally, the sample was filtered through 0.45 μm cellulose acetate syringe filter (Sterlitech Corporation, Kent, WA, USA), evaporated to 250 μL in a fume hood, and subjected to HPLC, FTIR, and GC-MS analysis to confirm biodegradation.

HPLC analysis of control dye RB 172 and its decolorized metabolites were performed with Waters 2690 instrument (Waters Limited, Hertfordshire, UK) equipped with C_{18}
column (symmetry, 4.6 × 250 mm). The isocratic method using the methanol with a flow rate of 0.50 mL min\(^{-1}\) for 10 min and UV detector set at 280 nm was used [13]. A total of 10 \(\mu\)L of dye RB 172 dissolved in methanol and its degradation metabolites were manually injected into the column and elution profile was observed. FTIR analysis was done in the mid IR region of 600–4000 cm\(^{-1}\) with scan speed 16 using the Shimadzu 8400S spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The samples prepared with spectroscopic pure KBr were fixed in the sample holder and analyzed [13]. The identification of metabolites formed after decolorization was carried out using a QP2010 gas chromatography coupled with mass spectrometry (Shimadzu Corporation, Kyoto, Japan). The ionization voltage was set at 70 eV and gas chromatography was performed in temperature programming mode with Restek column (0.25 mm × 30 m long). The initial column temperature was set at 40°C for 4 min, then increased linearly at 10°C/min to 270°C, and held for 4 min. Injection port temperature was 275°C and mass interface was maintained at 300°C. The helium with a flow rate of 1 mL/min was used as carrier gas for 30 min of run time [20].

2.7. Extraction and Activities of Biotransformation Enzymes. Extraction of enzymes after decolorization of dye RB 172 by \textit{P. rettgeri} strain HSL1 and control medium (without dye) was carried out as per the procedure described earlier [21]. The bacterial cells were separated by centrifugation (7500 × g for 15 min, 4 ± 0.2°C) and the resultant supernatant was considered test sample for determination of extracellular enzyme activities. The separated bacterial biomass was resuspended in 50 mM potassium phosphate buffer (pH 7.4), homogenized, and sonicated by giving 7 strokes of 30 s each for 2 min interval based on 50 amplitude output at 4 ± 1°C (Sonics-VibraCell ultrasonic processor). These sonicated cells were again centrifuged (7500 × g for 15, 4 ± 0.2°C) and the supernatant was used as a source of intracellular enzymes. Similar protocol was followed to quantify the enzyme activities of control medium. Enzyme extracted from the culture medium without adding dye was considered control.

Activities of oxidoreductive enzymes such as laccase, azoreductase, and NADH-DCIP reductase were assayed spectrophotometrically at room temperature (30 ± 1°C). Laccase activity was determined by measuring the oxidation of ABTS at 420 nm (\(\varepsilon_{420}\) nm = 36000 (M cm\(^{-1}\))) [22]. Determination of azoreductase activity was performed as per the procedure of Chen et al. [23], while NADH-DCIP reductase activity was assayed as reported previously [24]. All enzyme activity assays were conducted in triplicate and average rates were calculated. The protein content was determined by the method of Lowry et al. with bovine serum albumin as the standard [25].

2.8. Toxicity Studies. Environmental risk assessment of dye RB 172 and its degradation metabolites accumulation in animals was assessed by acute toxicity test with freshwater organism \textit{Daphnia magna} as described elsewhere [11, 26]. The dye treated sample with \textit{P. rettgeri} strain HSL1 was centrifuged (7500 × g for 20 min, 4 ± 0.2°C), supernatant-collected, and sterilized by passing through 0.45 μm cellulose acetate syringe filter. The clear filtrate (100 mL) was taken into a 250 mL Erlenmeyer flask and five 24 h old neonates of \textit{D. magna} were added. The tests were performed at 20 ± 0.2°C for 48 h in the absence of light and number of immobile organisms was counted after exposing to light for 20 seconds.

Toxicity of dye RB 172 and its degradation metabolites to plants was analyzed at room temperature on two kinds of economically important agricultural crops: \textit{Sorghum vulgare} (monocot) and \textit{Phascolus mungo} (dicot) as described earlier [13]. Briefly, ten seeds of both plants were daily irrigated with 10 mL each of RB 172 (50 mg L\(^{-1}\)) and its degradation metabolites (50 mg L\(^{-1}\)). Length of shoot, root, and seed germination (%) was recorded after 13 days. Both the tests were conducted in triplicate with control in distilled water.

2.9. Statistical Analysis. One-way ANOVA was analyzed and Tukey-Kramer multiple comparison test was performed with GraphPad Prism to determine the significance of the parameter studied.

3. Results and Discussion

3.1. Decolorization of Textile Dye RB 172 in AWWB Medium. The preliminary investigation on WB as growth medium for decolorization of RB 172 by \textit{P. rettgeri} strain HSL1 was carried out under microaerophilic conditions. The result of the UV-vis spectral analysis (400–800 nm) of the dye and its colored medium suggested that the \textit{P. rettgeri} strain HSL1 treated medium (20 h) showed enhanced reduction in the absorbance indicating dye decolorization (Figure 1(a)). The removal of colour indicates that WB can be utilized as growth medium for decolorization of dyes which signifies the low-cost treatment approach. It is reported that \textit{Providencia} sp. SRS82 could decolorize textile triazo dye Acid Black 210 in nutrient medium [20]. In addition, degradation of textile effluent by a developed bacterial consortium consisting of \textit{Providencia} sp. SDS and \textit{Pseudomonas aeruginosa} strain BCH has been reported in yeast extract medium [21]. As per our best knowledge, this is the first report showing the decolorization of textile azo dye by \textit{Providencia} sp. using WB as growth medium under submerged conditions.

For the successful operation of biological wastewater treatment systems, the impact of aeration that provides oxygen for bacterial growth and stimulates its contact with medium substrates should be properly analyzed. Monitoring the efficiency under microaerophilic condition, \textit{P. rettgeri} strain HSL1 showed >99% decolorization dye RB 172 (50 mg L\(^{-1}\)) within 20 h at 30 ± 0.2°C, whereas aerobic condition achieved only 12% performance within the same time and even 18% in 24 h (Figure 1(b)). These results indicate that aerobic condition strongly inhibited the decolorization of dye RB 172. Similar findings were reported in a previous study, where \textit{Pseudomonas} sp. SUKI exhibited higher decolorization rate of reactive azo dye Red BLI under microaerophilic condition whereas aerobic incubation showed only the growth but no decolorization [27]. It is reported that azoreductase
is the key enzyme responsible for breakdown of azo bond of azo dyes and presence of oxygen normally inhibits the azo bond reduction [28]. Furthermore, aerobic condition may dominate the use of NADH and impedes the electron transfer from NADH to azo bonds resulting in the decreased decolorization performance [29]. Hence, in this study, further decolorization of azo dye RB 172 was carried out only in microaerophilic conditions.

3.2. Optimization of Decolorization Conditions. To scale up the decolorization process and provide an affordable treatment technology for textile wastewater, the optimization of decolorization conditions such as growth medium pH, incubation temperature, and dyes concentration was carried out. Result of the study demonstrated that bacterial strain could decolorize the dye at broad range of pH; however, the optimum pH was found to be 7.0 (Figure 2(a)). A significant decrease in the decolorization performance was observed at lower pH (3–5) and higher pH (9–12). The transport of dye molecules across cell membrane has been known to govern by pH of the medium, which is considered the rate limiting step in decolorization process [30].

The enhanced and maximum decolorization activity of dye RB 172 by bacterial culture was observed at 30±0.2°C temperatures within 20 h of incubation in microaerophilic condition (Figure 2(b)). Further increase (37, 40, and 50°C) or decrease (20°C) in incubation temperature resulted in reduction in the decolorization performance. Effect of temperature on biodegradation of dyes might be associated with the microbial growth and enzymatic status of bacterial culture at respective conditions which determines its degradation abilities. Agrawal et al. reported that Providencia sp. SRS82 exhibited maximum dye decolorization activity for dye Acid Black 210 at 30°C temperature whereas lower and higher temperature than optimum have considerably decreased its decolorization rates [20].

The ultimate aim of wastewater treatment is to reduce the concentration of dyes. Result of the decolorization study at various concentrations (50–250 mg L⁻¹) showed that complete and rapid performance was observed at 50 mg L⁻¹ within 20 h by *P. rettgeri* strain HSL1 (Figure 2(c)). The decolorization efficiency of bacterial culture was found to be decreased at dye concentration above 100 mg L⁻¹. It has been suggested that the concentration of dyes can influence the decolorization efficiency of bacteria due to the toxic effect imposed at higher concentrations [31].

3.3. Dye Mineralization Analysis. The efficacy of textile wastewater treatment is determined by the mineralization of dye molecules in terms of decrease in COD and TOC contents [32]. Result of the dye decolorization by *P. rettgeri* strain HSL1 at optimum conditions, that is, WB medium pH 7.0, incubation temperature 30±0.2°C, 50 mg L⁻¹ of dye concentration, and microaerophilic incubation, suggests that the complete decolorization with significant reduction in COD (85%) and TOC (52%) was observed within 20 h (Table 1). These decreased magnitudes of analyzed parameter suggest the applicability of WB medium for growth of *P. rettgeri* strain HSL1 and their use in mineralization of azo dye RB 172. Additionally, the remained agricultural residues after preparation of WB medium could be used as low-cost adsorbent for dye removal and subsequent degradation by SSF [33]. But the SSF based methods work better with water soluble dyes as dye must adsorb on solid substrate prior to degradation. This signifies the importance of our work over several studies where biodegradation of textile dye was carried out using nutrient medium [34, 35]. It is well known that cost of growth medium used has strong influence on overall bioremediation economics. The market price of wheat bran displayed on the world’s biggest online commerce company http://www.alibaba.com/ is US $154–162/metric ton, while the cost of mostly used defined growth

![Figure 1: (a) UV-vis spectral analysis of control dye RB 172 and its decolorized broth by *P. rettgeri* strain HSL1. (b) Percentage of dye decolorization under microaerophilic and aerobic conditions. Data point represents the mean of three independent replicates; ±standard error of mean (SEM) is indicated by error bars.](image-url)
medium nutrient broth is US $5000–20000/metric ton. This huge difference in price of wheat bran and nutrient medium signifies the importance of our work for designing affordable biological wastewater treatment processes.

3.4. Enzyme Analysis. Results of the enzyme activity analysis suggest that *P. rettgeri* strain HSL1 possesses laccase, azo reductase, and NADH-DCIP reductase enzyme system in control cells. On the other hand, significant induction in the activities of laccase (60%), azo reductase (159%), and NADH-DCIP reductase (88%) from decolorized medium cells indicates its active involvement in breakdown of dye RB 172 (Table 2). Higher induction in the activity of azoreductase as compared to laccase highlights the dominance of reductive enzymes in decolorization process. Lade et al. reported the involvement of azo reductase in enzymatic cleavage of azo dye Trypan Blue by bacterial consortium [18]. Additionally, the roles of oxidoreductive enzymes in the decolorization of reactive azo dye Red HE3B have also been characterized in Providencia sp. SDS [21].

3.5. Biodegradation Analysis. The HPLC analysis of control dye showed the presence of one major peak at retention time of 2.702 min and three minor peaks at retention times of 2.125, 2.801, and 3.394 min (Figure 3(a)). After the dye decolorization process, the disappearance of peaks as seen in case of the control and the formation of completely different three major peaks at retention times of 2.521, 3.241, and
3.6. Toxicity Analysis. The treated textile wastewaters are being commonly discharged into the environmental sinks. Hence, it becomes essential to assess the risk of treated
Azo bond cleavage by azoreductase

\[
\text{[A]} \quad \text{4-(Ethenylsulfonyl) aniline (MW = 183, m/z = 183)}
\]

\[
\text{[I]} \quad \text{3,4-Diamo-6-[4-(1-amino-2-oxo-propyl)-phenylazo]-methyl]-5-hydroxy-naphthalene-2,7-disulfonic acid (MW = 523.54)}
\]

\[
\text{[B]} \quad \text{1-Amino-1-(4-aminophenyl) propan-2-one (MW = 164, m/z = 165)}
\]

**Figure 5:** Proposed metabolic pathway for the biodegradation of dye RB 172 by *P. rettgeri* strain HSL1.

wastewaters for animal and plants with high accuracy and ecological relevance. The acute and phytotoxicity assays are advocated as essential tools for addressing these issues [26, 35]. Acute tests with *D. magna* have been suggested as a primary screening method for the evaluation of lethal toxicity of chemicals to mammals and humans [37]. Result of the acute test showed 100% mortality of *D. magna* in untreated dye RB 172 (50 mg L\(^{-1}\)) solution suggesting the toxic nature of dye (Table 3). The acute toxicity is assumed to occur in test organisms when the accumulated dye content equals a critical concentration. In contrast, the treatment of dye RB 172 with *P. rettgeri* strain HSL1 was sufficient to completely detoxify the dye as no mortality of *D. magna* was observed in treated samples.

Result of the phytotoxicity analysis revealed inhibition of germination for each seed of *S. vulgare* and *P. mungo* by 70 and 60%, respectively, treated with 50 mg L\(^{-1}\) of dye RB 172 solution (Table 4). However, near about 90% germination was observed in both the seeds irrigated with dye degradation metabolites. Additionally, good elongation of shoot (9.2 and 10.2 cm) and root (3.6 and 4.1 cm) lengths for *S. vulgare* and *P. mungo* respectively, was observed in dye

**Table 3:** Mortality of *D. magna* exposed to dye RB 172 and its culture supernatants obtained after degradation by *P. rettgeri* strain HSL1.

| Samples                  | Mortality (%) |
|-------------------------|---------------|
| Distilled water         | 0 ± 0.0       |
| RB 172 (50 mg L\(^{-1}\)) | 45 ± 2.0     |
| Treated dye medium      | 0 ± 0.0       |

Values are mean of three experiments ± SD.
Metabolites: 4-(Ethenylsulfonyl) aniline [I]

Retention time (min): 19.54

m/z: 183

Metabolites: 1-Amino-1-(4-aminophenyl) propan-2-one [II]

Retention time (min): 23.10

m/z: 165

Table 4: Phytotoxicity of the dye RB 172 and its metabolites obtained after degradation by P. rettgeri strain HSL1.

| Samples            | S. vulgaris | P. mungo |
|--------------------|-------------|----------|
| Germination (%)    | Shoot length (cm) | Root length (cm) | Germination (%) | Shoot length (cm) | Root length (cm) |
| Distilled water    | 100         | 9.5 ± 0.5 | 3.8 ± 0.3 | 100          | 10.4 ± 0.4 | 4.5 ± 0.2 |
| RB 172 (50 mg L⁻¹) | 30          | 4.5 ± 0.2* | 2.2 ± 0.1* | 40           | 5.8 ± 0.2* | 2.1 ± 0.3* |
| Degradation metabolites | 90      | 9.2 ± 0.4 | 3.6 ± 0.4 | 90           | 10.2 ± 0.3 | 4.1 ± 0.2 |

Values are mean of three experiments ± SE.

Seeds germinated in dye are significantly different from control (distilled water) at *P < 0.001 by one-way analysis of variance (ANOVA) with Tukey-Kramer comparison test.

degradation metabolites grown plants. The strong influence of physiological characteristics in untreated dye grown plants suggests that dye RB 172 has toxic effect on plants as it inhibited germination and affected shoot and root elongation. The overall findings of the degradation study and toxicity analysis demonstrated that P. rettgeri strain HSL1 is not only able to decolorize the dye RB 172 but also completely detoxify it. This suggests the future application of P. rettgeri strain HSL1 for low-cost biodegradation as well as detoxification of azo dye contaminated wastewaters.

4. Conclusions

Wheat bran was successfully utilized as the growth medium for degradation of dye RB 172 by using P. rettgeri strain HSL1. A real market cost analysis of WB with defined growth medium nutrient broth suggests that WB could be used as a low-cost growth medium for bioremediation processes. The low-cost wheat bran medium, rapid degradation, and complete detoxification of model azo dye by P. rettgeri strain HSL1 revealed an economical and ecofriendly approach for designing azo dye containing wastewater treatment technologies. However, further studies are required to explore the use of WB medium for growth of bacteria and their use in the treatment of real textile effluent at reactor scale, which is an objective of our future research.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors’ Contribution

Harshad Lade performed the actual work and wrote the paper. Sanjay Govindwar and Diby Paul supervised the work.

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Research Article

Monitoring Pesticide Residues in Surface and Ground Water in Hungary: Surveys in 1990–2015

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Over 2000 surface, ground and raw drinking water samples have been analyzed in the frame of different monitoring projects in Hungary and watercourses in neighboring countries between 1990 and 2015. Effects of pesticide contamination on ecological farming and drinking water supply have been assessed. Main water pollutant ingredients of agricultural origin in Hungary are herbicides related to maize production. After EU pesticide re-registration, diazinon, atrazine, and trifluralin gradually disappeared as contaminants. High levels of water soluble pollutants (e.g., acetochlor) in surface water result in temporarily enhanced levels in raw drinking water as well. Extreme levels observed for herbicide residues were of agrochemical industrial origin.

1. Introduction

The widespread use of pesticides for agricultural and non-agricultural purposes has resulted in the presence of their residues in surface and ground water resources. The physicochemical properties of pesticide compounds, particularly their solubility in water and organic solvents, characterized by their octanol-water partition coefficients, determine their character of leaching into surface and ground waters [1]. Depending on their chemical stability, these substances may undergo decomposition processes; therefore, not only active ingredients but their metabolites may also occur as contaminants [2–4]. Most pesticides released into the environment are regarded as toxic substances, and newly emerging toxicological interactions have also been identified (mutagenicity, carcinogenicity, hormone modulant effects of environmental endocrine disruptor chemicals (EDCs), immunomodulant effects). Unfortunately not only pesticide residues but also other organic micropollutants (pharmaceuticals, personal care products, etc.) deteriorate water quality. Surfactants are common additives in agrochemical formulations to improve water solubility and uptake of the active ingredient and enhance its pesticide efficacy. Residues of surfactants are often detected in the environment; thus they can influence the effect of pesticide active ingredients. Recent studies indicate that combined toxicity of pesticide residues with other chemicals in agricultural use (e.g., adjuvants, detergents) has to be considered.

Contamination occurs not only due to current use of agrochemicals but also due to leaching of persistent ingredients from soil. Pesticide contamination of surface water in a particular region depends on several factors, such as closeness of crop fields to surface water, characteristics of surrounding fields (soil, grassland, slope, and distance to water bodies), and climate conditions (temperature, humidity, wind, and precipitation). In consequence, pesticide residues are being reported as common organic contaminants worldwide in surface waters and other environmental matrices [5–7]. Yet, pesticide residues may originate from urban pesticide application (e.g., roads, rails, urban, and family gardens): pesticides coming from urban areas are estimated to be responsible for as high as 30–50% of the total annual load. According to a study [8] the load of glyphosate from urban drainage system during rain events can exceed amounts of this herbicide originated from agricultural areas. Fast runoff from hard surfaces (e.g., urban roads) can lead to a fast increase of concentration shortly after the beginning of rainfall as it has been reported for glyphosate [8].
The presence of pesticides in water is regulated through different directives, including the Ground Water Directive [9], the Drinking Water Directive [10] and the Water Framework Directive [11], modified later several times [12, 13], setting a maximum concentration of 100 ng/L for individual pesticides and their degradation products, and 500 ng/L for total pesticide residues present in a sample. Currently environmental quality standards (EQSs) exist for 45 compounds or groups of compounds. There were also extensive programs to monitor water status of in European rivers and in ground waters which focused mainly on polar organic persistent pollutants. Aminoglyphosate is a common problem in Europe, but due to its good water solubility its extraction from water is difficult and its low detectability often has to be enhanced by derivatization. Despite of numerous analytical procedures published in the literature, determination of glyphosate is often missing from monitoring schedules. For glyphosate, only most recent LC tandem MS methods using electrospray ionization (LC-ESI-MS/MS) meet the Maximum Residue Level (MRL) by the EU for pesticide residues in drinking water [10], but the instrumentation demands of these methods are substantial. In contrast to these labor-intensive analytical procedures, enzyme-linked immunosorbent assays (ELISAs) allow selective and sensitive determination of glyphosate without sample preparation, but in these procedures AMPA is not detected. Due to difficulties in analysis large scale monitoring of glyphosate residues are seldom reported.

1.1. Pesticide Contamination Status in Surface Water in Hungary. Pesticide contamination presents a risk to the drinking (raw and tap) water supply and poses a problem via irrigation to ecological agriculture hindering pesticide-free farming. As the proportion of water wells obtaining raw drinking water from surface water resources is high in Hungary, especially in the region of Miskolc and along river Danube, the presence of pesticide residues in surface water bodies involves the risk of contamination of drinking water. The efficacy of pebble filtration on removal of microbial and organic micropollutants is low and effects of subsequent steps in classical drinking water treatments (e.g., chlorination, ozonization, and irradiation) do not effectively remove these pollutants. Persistent residues in soil exert their effects for long periods, and pesticides in surface and ground water may result in unintended exposure to residues through irrigation water.

Although surface water is regularly monitored for pesticides since 1976 in Hungary [17], earlier data are generally poor, and numbers of target compounds included were low. At the very beginning only some of persistent organic pollutants (POPs, e.g., organochlorine compounds) were regarded as problematic substances, in addition certain selected triazene, organophosphate, and chlorophenoxy acid type compounds were only involved in the monitoring schedule. At that time surface water analysis for residues mainly focused on compounds belonging to one or two different classes of pesticides that are amenable to GC. Later chloroacetanilides were also included, but some compounds were still omitted. Before long, given active ingredients (e.g., atrazine) proved to be persistent under certain conditions and occurrence of often applied water soluble ingredients (e.g., acetochlor) has also been observed [17]. The Hungarian Plant Protection Service has analyzed about 2000 surface water samples between 1994 and 2000 and found 5–50% of the samples containing pesticide residues. Atrazine was the most frequently found water contaminant that was detected in 6% of samples at levels of ~100 ng/L. It was followed by acetochlor, another herbicide active ingredient applied for corn crops: 4% of water samples contained acetochlor. Diazinon and hexachlorocyclohexane (HCH) were often determined at low concentrations. Propisochlor and metalachlor occurred less frequently, but their maximum concentrations fell between 10 and 100 ng/L.

The presence of plant protecting agents in raw drinking water was investigated by the end of the last century in Hungary [18]. Traces of pesticides have been detected especially in waterworks using surface and bank filtered water. About 44% of surface samples contained pesticide residue above limit of quantification and among these samples 13% exceeded the level of 100 ng/L. This maximum concentration for individual pesticides and their degradation products present in the samples have been established by Directive 98/83/EC [10] on the quality of water intended for human consumption. Among pollutants low levels of diazinon, high levels of atrazine (5700 ng/L) and in a single case prometryn reaching the level of 3220 ng/L were found. Chlorophenoxy acid type herbicides (2,4-D, dichlorprop, MCPA) were also often detected (8–15%) at levels up to 680 ng/L. These identified risk cases for
drinking water led to temporary or permanent closure of certain water wells.

These early detection cases prompted us to take part in different monitoring projects launched in the last fifteen years to obtain information on extent and distribution of pesticide pollution in Hungary. At the beginning of our work about 400 pesticide active ingredients were authorized in Hungary, and then the number of registered pesticide active ingredients was drastically reduced upon the pesticide re-registration process with regulations on pesticide usage in the European Union (EU) in 2005. This paper summarizes the results of these monitoring projects on pesticide contamination in surface and ground water resources in urban and agricultural areas in Hungary.

2. Experimental

In this work, a total of 49 pesticide residues and degradation products, belonging to different chemical classes, were monitored in Hungary. Water samples have been collected in the frame of seven monitoring projects in over twenty sampling campaigns between 1990 and 2015. Each sampling campaign had defined objectives and corresponding sampling regimes. In certain sampling campaigns, soils on cultivation fields were also sampled. Selection of target pesticides was done on the basis of their use and persistency. Determination of the selected analytes was performed using solid phase extraction (SPE) of water samples (1000x concentration factor) followed by GC-MS with or without derivatization [19], while determination of neonicotinoid insecticides was carried out by HPLC and glyphosate was measured by ELISA [20].

2.1. GC Analysis. Analytical sample preparation and GC-MS procedure was a multiresidue pesticide analysis method applied by survey authorities in Hungary [21] and modified and validated in our laboratory [22]. Acidic ingredients, for example, chlorophenoxy acid type herbicides, were eluted from graphitized carbon black SPE cartridges in a second fraction and were then subjected to derivatization to silyl esters using t-butylidimethylsilyl N, N-dimethylcarbamate as silylating agent and trifluoroacetic acid catalyst [19]. GC-MS analysis was performed on a Varian Saturn 2000 workstation equipped with a Varian CP 8200 autosampler (Varian Inc., Walnut Creek, CA, USA). Quantification of the selected pesticides was performed using matrix-matched calibration. The estimated values of the limits of detection (LODs) were in the range 0.4–5.5 ng/L.

2.2. HPLC Analysis. Determinations of neonicotinoid type pesticide active ingredients were performed on Younglin YL9100 HPLC system equipped with YL9150 autosampler (Younglin Co., Anyang Korea). Compounds were separated on a C18 column (Agilent Extend-C18, 150 mm × 4.6 mm i.d., 5 μm) equipped with an Agilent Guard column (12.5 mm × 4.6 mm i.d., 5 μm) at 40 degrees. UV detector signals were recorded at λ = 252 nm and λ = 269 nm. Eluent flow rate was 1.0 mL/min during the isocratic elution until 8 minutes (70:30 = A : B eluents, A = 90% water: 10% MeOH, B = MeOH). External calibrations based on the results for standard solutions (Pestanal) were used for quantification. If low concentration ranges required, HPLC-MS/MS measurements were carried out on a Bruker AmaZon SL ion trap instrument (Bruker AXS GmbH, Karlsruhe, Germany) operated in the positive electrospray ionization mode, upon SPE preparation of samples. Retention times were 2.42 min for thiamethoxam and 3.38 min for its decomposition product, clothianidin. LOD determined with standard solutions and with UV detector lied at 10 μg/L. External calibration based on the results obtained for 12 standard solutions in the range of concentrations between 10 μg/L and 150 mg/L. Determinations obtained upon SPE (Sep-Pak C18) with standard solutions and with MS/MS detector allowed LODs of 4 ng/L for thiamethoxam and 17 ng/L for clothianidin. Calibration solutions were prepared from a stock solution by dilution with water.

2.3. ELISA. As desirable low LODs for glyphosate and AMPA were not achieved even after their labor-intensive extraction [23] followed by derivatization prior to GC-MS analysis, for determination of glyphosate in ground and surface water, an immunoanalytical method, the commercially available ELISA method (PN 500086) by Abraxis LLC (Warminster, PA, USA) [24], was applied. Measurements [20] were carried out in 96-well microtiter plates according to manufacturer instructions. Comparative results with LC-MS or LC-MS/MS [25, 26] demonstrated the reliability of this competitive ELISA method; therefore, we have used it in our monitoring studies. The main drawback of the method is that it does not detect AMPA; therefore, due to the fast decomposition of glyphosate its environmental occurrence can be underestimated. On the other hand a comparative study [27] has established that immunoassay overestimated glyphosate concentration in some cases and detected a trace level in a sample deemed uncontaminated by LC-MS/MS.

3. Results

3.1. Nationwide Survey of Pesticide Residues in Surface Water in Hungary. A national survey (Project OMF 02193/1999; Monitoring of pesticide residues in surface and ground water, 1999–2002) was launched together with the National Service for Plant and Soil Protection (NSPSP) to assess chemical contamination levels in water bases in Hungary, to explore the points of vulnerability, and to identify pesticide residues in surface and ground water throughout the country. An additional aim was to inspect whether chemical loads on the environment decreased due to the introduction and implementation of integrated pest management (IPM) practices and the spread of ecological (organic) agriculture and to indicate whether pesticide contamination occur as point source or diffuse contaminants. Thus, 332 surface and raw drinking water samples were collected at 90 sites in Hungary (see Figure 1). The overall numbers of water samples collected and analyzed were 118, 119, and 95 in 2000, 2001, and 2002, respectively. Among these samples 24, 16, and 11 were
tap-water samples provided by Wedeco Waterworks Hungary or collected in the region of Vác in 2000–2002, respectively. In the first year of the survey (2000) 32% of water samples were found to be contaminated mainly by acetochlor and atrazine up to the level of 10000 ng/L (!) and prometryn have also been found at lower concentrations (1–10 ng/L). Two point contamination sources of industrial origin were identified in the region of Balatonfüzfő and the Northern Hungarian Chemical Works (Sajóecseg) (see Figure 1, sites 1 and 26). In 2001, 58% of samples contained pesticide residues above the LODs. Earlier mentioned ingredients showed similar pattern; 36% of samples were polluted by atrazine and among them 3% are at concentrations above 1000 ng/L, whereas the same ratios for acetochlor were 16% and 6%. Thus, acetochlor occurred less frequently, but higher concentrations have been determined. Prometryn was found in 7% of the samples at levels of 100–10000 ng/L. Among other pollutants trifluralin (10–10000 ng/L), metribuzin (100–1000 ng/L), and terbutryn (10–1000 ng/L) were detected in 1–3% of samples. Although diazinon was often (36%) found, its levels were usually low (10–100 ng/L). Regarding seasonal variation of residues it is worthy of note that one-third of samples polluted by atrazine and/or diazinon were collected prior to pesticide application, indicating persistence of these active ingredients under appropriate circumstances. The last year of the project (2002) focused on contaminated areas; therefore, 91% of collected samples contained one or more pesticide active compound. Maximum levels for atrazine and acetochlor remained high (over 15000 ng/L and 46000 ng/L) and contamination rates for these ingredients were 44% and 31%, respectively. Prometryn was detected in 18% of samples up to 1270 ng/L. Frequently found diazinon (65%) at levels 10–100 ng/L and in 3% of samples terbutryn (467–1671 ng/L) were determined.

Regarding raw drinking water samples there was only a single case when acetochlor has been detected during the first two years. However, in the autumn of 2002, acetochlor contamination in raw drinking water was observed in the region of Vác near river Danube. Its concentration in raw drinking water occurred to be near 100 ng/L, sometimes exceeding the MRL for drinking water in the EU. To our surprise simultaneously collected surface water samples from river Danube contained similar concentration of this ingredient (80 ng/L). Acetochlor contamination of raw drinking water was also detected in Verőce (34–64 ng/L), but here the levels remained under MRL. As the contamination levels in the river were not extremely high, results indicated the pesticide content passed through bank filtration and water treatment (e.g., chlorination) and occurred at unmodified levels in tap water.

3.2. Assessment of Point Source Pesticide Contamination in Hungary. On the basis of results obtained in the nationwide survey, regions of identified point source contamination sites were monitored (Project KvVM-KAC; Revision of pesticide active ingredients regarding environmental assessment and monitoring results, 2003). Sampling was carried out mainly near Lake Velence and in two regions of Lake Balaton (Balatonfüzfő and Tihany). This project, supported by the Hungarian Ministry of Environment and Water, was also

Figure 1: Sampling sites in the national survey between 1999 and 2002.
connected to the revision of pesticides considering environmental aspects and pesticide residue monitoring data. In the region of Balatonfüzö extensive sampling was performed (62 samples) in order to assess the extent and severity of earlier detected point source contamination of industrial origin. Additional 21 sites at Lake Balaton, 14 sites at Lake Velence, and 11 sites in Budapest and other regions were sampled. Surface and raw drinking water samples were collected at 80 sites in May and at 28 additional sites in June and August, 2003. Sampling was repeated at polluted sites in June and/or August. Thus, overall 135 surface and raw drinking water samples were analyzed during the project.

The contamination rate was found to be as high as 61%, and in accordance with earlier results, surface water samples collected in the region of Balatonfüzö contained high or extremely high levels of atrazine and acetochlor. Maximum concentration of atrazine was 8240 ng/L and 7540 ng/L in surface water and ground water, respectively. The corresponding values for acetochlor were found to be 13950 ng/L and 10070 ng/L, respectively. In addition, acetochlor could be measured in 56% of the tap water samples reaching the level 1075 ng/L. Lower levels of prometryn (up to 1025 ng/L) and terbutryn (up to 605 ng/L) have also been found. The quality of effluent waters originated from the industrial site of Nitrokémia Chemicals Works was of high concern, as contaminated water bodies flow through basins and ponds into stream Séd and then reach Lake Balaton. Concentrations of atrazine and/or acetochlor in these water courses were in the range of 2000–6000 ng/L, and sometimes exceeded the level of 10000 ng/L. Additional 18 sites in the neighborhood showed higher levels for acetochlor probably due to its leaching from contaminated soil around the area of Nitrokémia Works.

Atrazine was not detected and diazinon occurred in a single case at a level of 538 ng/L. In the region of Tihany, the highest concentration was found to be 424 ng/L in surface water, 359 ng/L in Lake Balaton, and unfortunately appeared in a drinking water sample at a level of 249 ng/L (Csopak). South from the point contamination source half of samples from Lake Balaton were contaminated by acetochlor reaching the maximum concentration of 1547 ng/L, whereas 332 ng/L was measured in Channel Sió. A similar pattern was observed at Lake Velence: 316 ng/L was determined in a surface water sample, whereas high contamination rates (88%) were observed in the lake itself with levels up to 702 ng/L and 2970 ng/L as a peak concentration. Comparing the concentrations determined in water samples collected at a certain polluted site in May, July, and September, the levels of acetochlor, terbutryn, and prometryn ingredients decreased and similar tendency have been usually observed for levels of atrazine. High levels for atrazine and acetochlor have been detected due to improper technology applied for washing pesticide containers (Papkeszi).

More than half (56%) of the raw drinking water samples collected in this polluted region near to Nitrokémia Works contained acetochlor above LOD. Contamination levels were in the range of 116 to 1075 ng/L.

3.3. Pesticide Residues Monitored in Surface Water at Regions of Ecological (Organic) Agriculture. Due to the high level of diffuse pesticide contamination observed in the national survey, a separate monitoring study (Project OMFB-00947/2005; Methods for detection of persistent organic soil and water pollutants in ecological cultivation, 2006–2008) was focused on persistent organic pollutants (POPs) and their effects on ecological (organic) cultivation. Between 2006 and 2008, 115 water resources, used as source for irrigation of organic crops, have been investigated. Thus, surface water and soil samples collected in April-May, August, and October at ecological farming sites (see Figure 2) were analyzed for persistent pesticide residues. In 2006, eight organic farms were sampled; later investigations focused on polluted problem areas (Szarvas, Karcag, and Tamási). In the first year, 24 ground water and 46 surface water samples were collected that were used as irrigation water. In 2007 and 2008, only polluted sites were sampled 21 + 12 surface and 6 + 6 ground water samples were measured.

Among the nine water pollutant pesticides, the most often occurring ones were trifluralin, atrazine, lindane, diazinon, acetochlor, and metolachlor. Diazinon could be detected in 25–80% of the samples at levels below 50 ng/L. About half of the samples contained atrazine (<250 ng/L) and/or trifluralin (<50 ng/L) ingredients. At two sites (Tamási and Karcag) high levels of atrazine (100–250 ng/mL) as point source contamination were detected probably due to drift from intensively cultivated field in the neighborhood.

In the second year, sampling focused on problem areas. Thus, surface and ground water samples were collected at three sites (Szarvas, Karcag, and Tamási). Three pollutants (trifluralin, lindane, and diazinon) were detected at low concentrations (5–15 ng/L) and traces of DDE (<3 ng/L) occurred in a single case. To our surprise atrazine or acetochlor has not been observed.

In the third year four ingredients (trifluralin, atrazine, diazinon, and metolachlor) were found and contamination rate lied around 62%. Contamination levels were between 1 and 20 ng/L, except for atrazine, which was determined in five water samples at higher concentrations (40–250 ng/L). Acetochlor has not been observed. Absence indicates that no illegal use was found regarding this often occurring water pollutant. Due to their leaching from soil diazinon, atrazine and trifluralin are common water pollutants in Hungary. These soil disinfectants and herbicides could be detected in 28%, 38%, and 48% of water samples, respectively.

In summary 56%, 28%, and 62% of water samples collected in the frame of project were found to be polluted by one or more pesticide active ingredients in 2006, 2007, and 2008, respectively. The same values for soils were 38%, 59%, and 41%, whereas the main soil pollutants were DDE, lindane, and trifluralin. Although surface water samples collected near ecological fields were not pesticide-free, residue levels found typically lower than that of intensively cultivated areas.

3.4. Comparison of Regional Pesticide Contamination in Intensive and Ecological (Organic) Agriculture. Levels of pesticide residues in surface water and soil are strongly related to
each other: organic micropollutants in surface or irrigation water may be absorbed in soil layers, and vice versa, soil contaminants may leach into surface water. Aiming at developing an ecotoxicology based monitoring system for soil micropollutants (Project NKFP_07_A4-MONTABIO; Complex monitoring system for analytical detection and biological evaluation of soil micropollutants for a sustainable environment, 2008–2010), environmental samples from were comparatively surveyed among cultivation sites of intensive and ecological farming at a regional (county level) survey. Within the three-year sampling campaign, 286 surface and ground water samples (2008: 81, 2009: 121, 2010: 84) collected in Békés county, Hungary (see Figure 3), were analyzed. The survey also included statistical assessment of the reliability of the sampling techniques, as well as the biological evaluation of aquatic ecotoxicity. Regarding the 14 sampling sites five industrial fields, four ecological fields, four intensively cultivated fields, and a pasture have been involved.

The survey revealed extensive contamination of both surface water and soil. During the entire sampling campaign, 139 water samples contained detectable contamination by one or more target compounds (contamination rate 49%). Acetochlor (22–3900000 ng/L) and diazinon (1–851 n/L) were the most frequently found water pollutants, metolachlor (1–56000 ng/L) was often detected, and atrazine (500–10000 ng/L) and trifluralin (800–900 ng/L) also occurred in 2008 and 2009. Diazinon was found only in 2008, and traces of terbutryn were also detected in two samples in the first two years of the project. In 2010 diazinon (18–651 ng/L) occurred again and higher rate of samples contained the most often detected acetochlor (22–6250 ng/L). Acetochlor was detected in 61% of contaminated water samples, 24% contained diazinon, 19% metolachlor, 16% atrazine, and 11% trifluralin. Some other ingredients (prometryn, dimethenamid, dimethirimol, and ethofumesate) occurred in 2–3% of contaminated samples. One-quarter of the soil samples contained traces of DDT (the insecticide banned in Hungary first in the world in 1968) or its decomposition products. Due to their very low water solubility, these substances do not appear in surface and ground water samples, but other soil pollutants occur as water pollutants as well. Most common soil pollutants were trifluralin at concentrations of 1.0 to 1800 ng/g and acetochlor and metolachlor at lower levels.

In 2008, low amounts of precipitation resulted in higher contamination rate, and about 67% of water samples contained one or more pesticide active ingredients. In the next year only in 18% of water samples pesticide residue has been found. In 2010 sampling was performed only at sites where pesticide residues had been detected earlier; therefore, the contamination rate was understandably higher (73%) than in previous years. About a quarter of the samples contained only low levels (below 50 ng/L) of residues. Concentrations observed were similar to values measured in the previous year in accordance with high amounts of precipitation that was characteristic for both years. Pesticide residue levels were beyond the values set by Ground Water Directive [9]. Sixteen percent of samples exceeded the maximum concentration of 100 ng/L for individual pesticides, and in 10% of samples over 500 ng/L were total pesticides present as an average for three years. The same ratios were 32% and 12% in 2010, respectively.
Point source contamination due to illegal pesticide deposit was explored in the region of Gyomaendrőd. Acetochlor, atrazine, and trifluralin were found in soil at alarmingly high concentrations (up to 590 ng/g, 632–782 ng/g, and 1440–1882 ng/g). Ground water samples were also highly contaminated. For example acetochlor concentrations were in the range of 1870–6250 ng/L, atrazine concentrations measured 465–4990 ng/L, and trifluralin also appeared as some other ingredients (terbutryn and metolachlor). In the last year of the project (2010), high amounts of ethofumesate were found in 3 water samples collected at Gyomaendrőd and 2 samples contained dimethenamid and dimethirimol. These pesticide active ingredients have not been detected earlier. Dimethenamid is a currently used chloroacetamide type herbicide, in contrast to dimethirimol which is a pyrimidinol type systemic fungicide banned in EU since 2002.

Half of water samples collected at industrial sites in Orosháza (County Public Road Service and glass factory) contained hydrocarbon contamination. Only acetochlor pesticide residue could be detected in water and due to high background in the organic layer no pesticide residues could be identified.

In the frame of the project a new derivatization technique was developed for the determination of chlorophenoxy acid type herbicides from water samples. This procedure was routinely applied for analysis of surface and ground water samples collected from Békés county in Hungary in the autumn of 2010. Nearly half of the water samples collected were affected by residues of 2,4-D (11–38 ng/L) above LOQ and it could be detected in 79% of the samples. In three highly contaminated samples amounts of 2,4-D (176, 907, and 1003 ng/L) have been determined. Some other ingredients belonging to this group (meprop, MCPA, dichlorprop, MCPB) have also been detected in ten samples collected at sites Gyomaendrőd and Orosháza county Public Road Service. 2,4,5-TP (fenoprop) has not been registered in Hungary and it is banned in the EU since 2004.

The concentration of glyphosate was also determined by ELISA method [24] in 42 surface and ground water samples collected from Békés county in Hungary. Half of the 42 surface and ground water samples collected in September 2010 were contaminated by glyphosate at concentrations of 540–980 ng/L. Exceedingly high glyphosate levels (nearly 1000 ng/L) were measured in five ground water samples and significant concentrations (540–760 ng/L) were determined in 16 cases (3 surface and 13 ground water samples). Three ecological samples and two surface water samples from a pasture were also found to be contaminated.

In a similar survey carried out at cultivation sites of spice paprika, environmental and food safety aspects of spice paprika cultivation and production were investigated (Project EU-FP7-SEC-2012-1-312631; Securing the spices and herbs commodity chains in Europe against deliberate, accidental, or natural biological and chemical contamination (SPICED), 2014–2016). The environmental and food safety of spice paprika production is of key concern in spice production in Europe. As imported spice paprika commodities are often found contaminated by the EU Rapid Alert System for Food and Feed (RASFF), domestic spice paprika production is required to be assessed for organic micropollutants, and
environmental pesticide residue contamination is also to be monitored. In field studies three different paprika producers (2 sites of each) practicing intensive cultivation mode and three organic farmers have been involved. Thus, 110 soil samples were collected in paprika cultivation fields at 9 locations, and 6 water samples near to fields have also been sampled that have been partly used for irrigation in each sampling regime (June and September). Soil contamination has been found only in samples collected from intensive cultivation fields. Trifluralin, tefluthrin, and DDT together with decomposition products (DDE and DDD) and in a single case chlorpyrifos were determined, whereas in some cases traces of diazinon and atrazine and in a single case metolachlor were detected but were not quantified. Trifluralin has been measured in 50% of water samples at levels 11–34 ng/L. Half of soils collected at intensively cultivated sampling sites has been polluted by tefluthrin, one of the most toxic pyrethroids used against soil pests. Surface water samples collected there have not contained this ingredient probably due to its low water solubility. Although DDT and its metabolite (DDE) or degradation product (DDD) appeared at high levels in soil samples collected at two sites, due to their low water solubility they have not occurred in surface water nearby and or in paprika harvested from these intensively cultivated fields.

3.5. Transnational Survey of Seasonal Pesticide Contamination in Rivers in the Carpathian Basin. To assess the extent of pesticide contamination carried by rivers, in given cases through national frontiers (Project HUSK/0901/2.1.2/0076; Agrowater, 2011–2013), samples collected from Danube, Tisza and Vág rivers, streams, Lake Balaton, and other surface waters and some of drinking water samples were analyzed. Samples were collected in February 2011 before pesticide application along the Danube River, and the same sites from Hainburg (Austria) through Bratislava-Komarno (Slovakia) to numerous sampling points in Hungary, Mohács being the most Southern point, were revisited for repeated sampling after pesticide application during a one-month period after the middle of May. Other sites in the catchment area (Tisza, Balaton, and Vág) and tap water have also been sampled. Monitoring was conducted at eleven sampling sites along the river in the winter and at 31 sampling sites in the summer (see Figure 4). Monitoring continued in 2012 and 2013, but sampling has been restricted to Danube River (Budapest). Sixteen surface water samples from Danube and 12 tap water samples were taken twice a week in May and June and four additional samples from Lake Velence in the middle of June in 2012. Similar sample collection from Danube has been performed in 2013, but sometimes it had to be cancelled due to flood in the middle of June. Therefore only twelve samples were analyzed in that year.

All surface water samples contained traces of some pesticide residues (trifluralin, alachlor, and chlorophenoxy acids) in February indicating their slow degradation and dissipation rate. Withdrawn ingredient, alachlor, could be detected only in the winter sampling regime at low levels (0.7–10.3 ng/L). In the summer sampling regime (May–June) the ratio of surface water samples that exceeded the maximum concentration of 100 ng/L for individual pesticides was 41%, and 18% of samples contained total pesticide residue above 500 ng/L. Regarding the ingredients and the typical levels results were
in accordance with those obtained for samples in Békés county earlier. Acetochlor was the most frequently found pollutant. It was present in all but one surface water samples collected in May and June and typically higher concentrations (75–711 ng/L) have been observed in May than in June (23–162 ng/L). Metolachlor was the second most frequently detected ingredient polluted 65% of samples collected and levels in Danube were 31–241 ng/L. No special pattern for pollutants’ concentrations could be observed along the river. Earlier often detected and banned persistent water pollutants also appeared in samples collected in May and June. Similarly to results found in 2011–2013, atrazine was detected in 13% of samples at levels 17–40 ng/L, in addition trifluralin (25%, 4–31 ng/L) and ethofumesate (19%, 12–27 ng/L) also often occurred. Less frequently diazinon (16%, 6–10 ng/L) and prometryn (10% 7–40 ng/L) were observed.

All but one drinking water samples contained acetochlor and it could be measured around the MRL for drinking water in EU (100 ng/L) in three tap water samples collected in May. In accordance with earlier results 2,4-D residues were often present, and 80% of samples contained it even in winter samples at levels of 0.24–3.81 ng/L. Its amounts were the highest in samples collected in June (56–186 ng/L). Low levels of other chlorophenoxy acid type herbicides (mecoprop, dichlorprop, and MCPA) have also been detected but their amounts could be quantified only in two cases (6%).

Samples were analyzed for glyphosate residues by the immunoanalytical method used previously [24]. It was applied for 18 surface water samples collected from the Danube River and Lake Velence in Hungary at 12 sampling sites in the middle of May 2011 and repeated at six sites along the Danube in October (see Figure 4). Glyphosate was detected only in a single sample collected at Lake Velence in May and in two samples from Danube in the autumn at levels near LOD, therefore, practically no contamination was found in 2011. Monitoring of glyphosate was restricted to Danube River at Budapest in 2012. Samples were taken twice a week in May and June from Danube and four additional samples from Lake Velence in the middle of June. Three of sixteen surface water from Danube contained glyphosate near LOD (120 ng/L), but in other cases presence of ingredient was detected. Levels (125–242 ng/L) were near LOD except for sample collected on 1st June, when 455 ng/L was measured. Similar contamination rate (75%) was determined for samples from Lake Velence with levels near LOD (180–228 ng/L).

Results in 2014 and 2015 (Project AD006; Assessment of (bio)chemical, biological main and side-effects of organic microcontaminants of agricultural origin, monitoring, and determination in environmental and biological samples, 2014–2016) showed a similar pattern seen in 2011, but acetochlor the earlier most frequently found pollutant has not been observed, in contrast to metolachlor that was present in 75% surface water samples collected in May and June (45–365 ng/L). No special temporal variation in time for metolachlor concentrations could be observed. Atrazine could be detected in 13% of samples at levels 17–40 ng/L, often occurred trifluralin (25%, 4–31 ng/L) and ethofumesate (19%, 12–27 ng/L). Less frequently were observed diazinon (13%, 6–10 ng/L) and prometryn (6% 7–40 ng/L).

The vast majority of surface water samples (92%) contained neonicotinoids below LOD, while the highest concentrations (10–41 μg/L) were measured from temporary shallow water bodies after rain events in early summer. Only thiamethoxam and its decomposition product clothianidin were detected among neonicotinoids. These levels are in agreement with recent findings reported for neonicotinoids as surface water polluting contaminants [28–31].

3.6. Ecotoxicological Analysis. Given surface water contaminants were subjected to targeted ecotoxicological analysis. Thus, special emphasis was given the combined toxicity and ecotoxicity of glyphosate and its formulating adjuvants, as well as to distribution and ecotoxic effects of neonicotinoid active ingredients. Although glyphosate presents lower acute toxicity than other herbicides, its widespread use and difficulties in detection [32] prompts cautious assessment for combination effects as well. It has been evidenced to cause toxicity and genotoxicity in aquatic organisms and amphibians [33] and teratogenicity in amphibians and birds [34] and has been shown to induce endocrine disrupting effects as well [35], the latter effect being highly synergized by polyethoxylated tallowamine (POEA) and other commonly used formulating agents in glyphosate-based herbicide preparations. As an immediate consequence of the above toxicological and ecotoxicological concerns and as these substances have proven to be persistent under typical application conditions [36], glyphosate and its metabolite AMPA are required to be regularly monitored in surface and ground waters. Combinational ecotoxicological effects were proven in our hands as well, on various aquatic organisms [37, 38]. Moreover, adjuvant enhanced cytotoxicity has been evidenced on cell lines of animal [39] and human [40] origin.

Our preliminary results indicate that a newly emerging pesticide class of neonicotinoids can be found in environmental water samples as well. Sporadically clothianidin was found in ponds near to maize and sunflower crops emerged from treated seeds. These compounds are used mainly as seed dressings, and the portions not uptaken by target crops contaminate the environment. They accumulate in soil [41] and due to their good water solubility they appear in water resources. As neonicotinoids exert systemic action, the active compounds are translocated and distributed throughout the entire plant; therefore, consumption of different parts of plants (pollen, nectar) could be harmful to insects. Novel ways of intoxication for bees have also been explored, that is, water collection from guttation liquid [42]. They appeared in potatoes [43] and high contamination rates were reported for fruits and vegetables, as well as honey samples [44]. Serious bee poisoning events and risk assessment of EFSA in January 2013 led the European Commission to the conclusion [45] that a high risk for bees cannot be excluded except by imposing further restrictions for two years involving withdrawal of authorization of neonicotinoids and ban of treated seeds for different crops. The restriction applies to the use of 3 neonicotinoid active ingredients (clothianidin, imidacloprid, and thiamethoxam) for seed treatment, soil
application (granules), and foliar treatment on crops attractive to bees, including certain cereals. Our findings prompted us to expand our investigations to these target compounds as well as to other polar pollutants amenable only by LC-MS analysis.

4. Discussion

Pesticides residues in surface waters have routinely been detected in nationwide studies [17–22]. The rate of contaminated (detectable) samples ranged between 2 and 51%. In the period of 1994–2000, the most common contaminants were atrazine (6%), acetochlor (4%), propisochlor (1.5%), metolachlor (1.5%), diazinon (1%), and 2,4-D (1%). Key contaminants were atrazine and to some extent isoproturon, being found in several cases at above 100000 ng/L. Results of the national survey between 1999 and 2002 and other studies on problem areas also indicated diffuse contamination of surface and ground water in Hungary. Surprisingly high contamination rate, 32–61%, was found in monitoring projects. Concentrations of various pesticide active ingredients and metabolites investigated in surface water and raw drinking water, along with their MS identification descriptors, are presented in Table 1.

Two point contamination sources of industrial origin were identified in the region of Balatonfüzfő (Nitrokémia Chemicals Works) and Sajóécseg (Northern Hungarian Chemical Works) connected to former pesticide producers. Atrazine and acetochlor were found in soils in Balatonfüzfő (Nitrokémia lpartelepek) at alarmingly high concentrations reaching 10–400 ng/g; therefore, the levels of these ingredients in surface waters in surroundings, for example, in the Séd stream, exceeded the level of 10000 ng/mL. Extremely high levels were measured around Sajóécseg not only for acetochlor, but occasionally concentrations for atrazine, prometryn, and terbutryn were above 1000 ng/mL in the same sample [22]. Sometimes concentrations in soil were as high as ingredient content in formulated pesticides. At these sites due to exceedingly high residue levels phytoremediation is impossible. Point contamination source due to illegal pesticide deposit has also been explored in Gyomaendrőd. Apart from these extremities typically more than half of surface and ground water samples contained one or more pesticide active ingredient.

Temporal alterations of residue concentrations have been characterized by bimodal pattern. Whereas pesticide contamination in soil samples appeared to be more uniform in time, contamination rates and levels in water are time dependent. As amounts of precipitation strongly influence leaching of pesticides, levels determined depend not only on pesticide application, but also on meteorological conditions. As expected, the highest levels of pesticide pollution appeared in water samples collected in late spring and autumn campaigns but rarely occurred in waters sampled in August.

Although high contamination rates have been found, but due to the improvements of analytical methods, low LODs can be achieved for most target compounds and trace levels of contaminants are detected. One of the minimum performance criteria for analytical methods applied for monitoring chemical pollutants corresponds to the limit of quantification (LOQ) According to the WFD, LOQs should be equal or less than 30% of the relevant Environmental Quality Standards (EQSs) [16]. Legally only concentrations measured above the MRL are significant and samples containing pollution below the MRL are regarded as pesticide-free by authorities. Independently from toxicological considerations for individual ingredients, MRLs for pesticide residues in drinking water and ground water in the EU have been set to a common standard value (100 ng/L). Directive 2013/39/EU [13] proposed maximum allowable concentrations (MAC) and annual average (AA) for levels of priority compounds and certain other pollutants in inland surface and other surface waters as EQSs. Values were set for a number of pesticides including alachlor, atrazine, simazine, diuron, isoproturon, chlorfenvinphos, chlorpyrifos, endosulfan, trifluralin, hexachlorocyclohexanes (HCHs), DDT, aldrin, dieldrin, endrin, and isodrin [12]. MAC values for some of detected water pollutants in Hungary are 700 ng/L and 2000 ng/L for alachlor and atrazine, respectively, but for trifluralin no MAC value is applicable. In our surveys, these levels have rarely been exceeded, only in the cases of point contaminations, where higher concentrations were determined for atrazine. In contrast to the above mentioned limits, pesticide-free means zero level of residues for the public, and it is often a source of confusion or contradiction between the authorities and civil society.

Pesticide application adversely influences the quality of surface water supplies, especially when water soluble ingredients are used. High levels of pesticide in surface water can lead to their occurrence in raw drinking water. According to a survey related to drinking waters published in 1998 [18], the levels of atrazine, diazinon, and prometryn in subsurface water exceeded the MRL by the corresponding harmonized EC Directive [9], effective in Hungary as well, set to 100 ng/L for residues of a given pesticide and 500 ng/L for all pesticide residues in drinking water. Our investigations explored vulnerable points of drinking water supply along the Danube and confirmed these earlier detections. Due to insufficient efficiency of bank filtration in certain cases, pesticide residues may contaminate raw drinking waters. Our investigations in 2002 indicated tap water pollution by acetochlor concerned to the most vulnerable water resource, namely, to pebble filtration wells in the region of Vác nearby river Danube. Similar contamination was found earlier in Island Buki [18] near to these sites, where the drinking water well has been closed and as we reported, contamination repeatedly occurred in 2011 as well.

Another important issue is to avoid or minimize pesticide contamination in ecological cultivation. Organic farming is not free from pesticide residues because of (i) drift, leaching from fields treated with agrochemicals nearby; (ii) pesticide residues in irrigation water; (iii) persistent pollutants from treatments prior to organic cultivation; (iv) illegal pesticide application. We have observed that pesticide contamination levels determined in organically cultivated fields are on the average one order of magnitude lower than in intensively cultivated fields.
Table 1: Target compounds, their chromatographic retention time, and characteristic molecule ions for MS identification and concentrations found in surface water samples.

| Pesticide active ingredient/metabolite | Retention times (min) | Quantitation ion (other ions) \(m/z\) | Concentration range as diffuse contaminant (ng/L) | Comment |
|---------------------------------------|-----------------------|----------------------------------------|-----------------------------------------------|---------|
| GC analysis                           |                       |                                        |                                               |         |
| Neutral and basic substances          |                       |                                        |                                               |         |
| Propachlor                            | 10.075                | 120 (176, 212)                        | —                                             |         |
| Trifluralin                           | 10.817                | 306 (264, 290)                        | 800–10000                                     | Soil contaminant as well |
| Phorate                               | 11.038                | 121 (75, 231, 260)                    | —                                             |         |
| Simazine                              | 11.490                | 201 (173, 186)                        | —                                             |         |
| Atrazine                              | 11.651                | 200 (215, 216)                        | 500–15000                                     | >15600 ng/L (in 2002) as point source; soil contaminant as well |
| Lindane                               | 11.888                | 181 (183, 217)                        | 5–15                                          | Soil contaminant |
| Terbuthylazine                         | 11.910                | 214 (173, 229)                        | —                                             |         |
| Diazinon                              | 12.183                | 179 + 137 (152)                       | 10–900                                        | Soil contaminant as well |
| Tefluthrin                            | 12.301                | 177 (197, 141)                        | —                                             | Soil contaminant |
| Pirimicarb                            | 12.690                | 166 (239, 72)                         | —                                             |         |
| DDMS                                  | 12.925                | 235 (237, 199)                        | —                                             |         |
| Dimethenamid                          | 13.000                | 154 (230, 203)                        | traces                                        |         |
| Dimethachlor                          | 13.007                | 134 (197, 210)                        | —                                             |         |
| Metribuzin                            | 13.070                | 198 (57, 199)                         | 100–1000                                      |         |
| Acetochlor                            | 13.139                | 224 (223, 174)                        | 20–6300                                       | 46000 ng/L (in 2002) and 390000 ng/L (in 2008) as point source; soil contaminant as well |
| Heptachlor                            | 13.305                | 272 (274, 100)                        | —                                             |         |
| Alachlor                              | 13.345                | 188 + 238 (160)                       | 1–10                                          |         |
| Prometryn                             | 13.364                | 241 (242, 184)                        | 100–10000                                     | >6100 ng/L (in 2003) |
| Dimethirimol                          | 11.420                | 166 (209)                             | traces                                        | >100 ng/L in a single instance (in 2010) |
| Propisochlor                          | 13.451                | 224, 162 (223)                        | 10–100                                        |         |
| Terbutryn                             | 13.624                | 185 (242, 226)                        | 10–1000                                       | >83800 ng/L (in 2003) |
| Ethofumesate                          | 13.761                | 207 + 161 (286)                       | 10–30                                         |         |
| Malathion                             | 13.871                | 173 (127, 125)                        | —                                             |         |
| Aldrin-R                              | 13.990                | 293 (66, 293, 65)                     | —                                             |         |
| Metolachlor                           | 14.049                | 162 (238, 240)                        | 1–56000                                       | Soil contaminant as well |
| Chlorpyrifos                          | 14.070                | 314 (316, 258)                        | —                                             | Soil contaminant |
| Triadimenol                           | 14.900                | 168 (128, 71)                         | traces                                        |         |
| Penconazole                           | 14.700                | 248 (159, 251)                        | —                                             |         |
| Pendimethalin                         | 14.710                | 252 (282, 253)                        | —                                             |         |
| Heptachlor Epoxide                    | 14.775                | 353 (355, 351)                        | —                                             |         |
| Endosulfan                            | 15.490                | 195 (339, 160)                        | —                                             |         |
| Hexaconazole                          | 15.700                | 214 (111, 97)                         | —                                             |         |
| DDE                                   | 15.865                | 318 (208, 282)                        | <3                                            | Soil contaminant |
| DDD                                   | 16.907                | 235 (237, 207)                        | —                                             | Soil contaminant |
| Dieldrin                              | 16.059                | 79 (79, 81, 277)                      | —                                             |         |
| Endrin                                | 16.617                | 279 (243, 245)                        | —                                             |         |
| DDT                                   | 18.059                | 235 (237, 165)                        | —                                             | Soil contaminant |
| Iprodione                             | 19.508                | 314 (315, 316)                        | —                                             |         |
Table 1: Continued.

| Pesticide active ingredient/metabolite | Retention times (min) | Quantitation ion (other ions) \( (m/z) \) | Concentration range as diffuse contaminant (ng/L) | Comment |
|--------------------------------------|-----------------------|---------------------------------------------|-------------------------------------------------|---------|
| **Acidic substances**                |                       |                                             |                                                 |         |
| MCP tBDMSa                           | 7.524                 | 199 (201, 125)                              | —                                               |         |
| DCP tBDMSa                           | 7.957                 | 219 (221, 93)                               | —                                               |         |
| Mecoprop tBDMSa                      | 9.208                 | 225 (271, 227)                              | 10–15                                           |         |
| MCPA tBDMSa                          | 9.453                 | 257 + 229 (211)                             | 5–300                                           |         |
| Dichlorprop tBDMSa                   | 9.584                 | 245 (247, 291)                              | 3–200                                           |         |
| 2,4-D tBDMSa                         | 9.882                 | 251 + 233 (279, 277, 281)                  | 10–1000                                         |         |
| MCPB tBDMSa                          | 10.226                | 281 (325, 253)                              | 10–20                                           |         |
| 2,4,5-T tBDMSa                       | 10.526                | 285 + 311 (267, 75)                         | —                                               |         |
| 2,4,5-TP tBDMSa                      | 10.648                | 201 + 199 (202)                             | —                                               |         |
| 2,4-DB tBDMSa                        | 10.910                | 219 (201)                                   | —                                               |         |
| **HPLC analysis**                    |                       |                                             |                                                 |         |
| Thiamethoxam                         | 2.42                  | 292 (211, 246)                              | 4–30                                            | 10–41 \( \mu \text{g/L} \) measured from temporary shallow water bodies |
| Clothianidin                         | 3.38                  | 250 (132, 168, 169)                         | 17–40                                           |         |
| **ELISA**                            |                       |                                             |                                                 |         |
| Glyphosate                           | —                     | —                                           | 500–1000                                        |         |

\(^a^\) Chlorophenoxy acids and the corresponding phenol metabolites have been determined by a parallel chromatographic method as t-butyldimethylsilyl esters or ethers (tBDMS).

Drift from intensively cultivated fields has been observed, but illegal pesticide use could not be assumed, as most often water pollutants, atrazine, and acetochlor were hardly detected. Trifluralin seems to be persistent in soil under appropriate circumstances and its dissipation is slow. From persistent compounds, the presence of DDT and its decomposition product DDE can be still detected in about half of the Hungarian soils at very low levels, and they exert a long term effect.

Our results confirmed that ecological fields could be contaminated via irrigation water; therefore, it should also be monitored especially in corn cultivation regions. Although withdrawal of some water pollutants (atrazine in 2007, diazinon in 2008 and trifluralin in 2009) probably improved water quality, the use of certain water resources as irrigation water in ecological farming should/have to be restricted.

As it was observed later in project MONTABIO, withdrawal of the above mentioned ingredients resulted in their gradual disappearance. Atrazine could be detected only in samples collected at Gyomaendrőd in 2010, while earlier it had been detected in samples from Békés and Orosháza. Trifluralin often detected as a soil pollutant has, due to its limited water solubility, quite long dissipation time. Therefore, it could be detected in water samples in all years between 2008 and 2010. Diazinon was often found in water samples collected in 2008, not detected in 2009, but in 2010 eight ground water samples contained this insecticide. They appeared even in 2011; thus their dissipation is slow.

Frequent occurrence and temporarily high levels of acetochlor, as well as metolachlor, might be related to their use instead of atrazine in Hungary. Detections of acetochlor in surface water probably contributed to its withdrawal in EU in 2012. The temporal pollution “plaques” of herbicide residues in rivers upon broad field application of herbicides pollute potential irrigation water sources and pose risk to the drinking water supply. Concentrations of acetochlor and metolachlor reported in this study are comparable to those found in the Danube River basin in Serbia (80 and 150 ng/L) [46]. In contrast to this Serbian study terbuthylazine was not detected in our surveys.

Atrazine was used predominantly as herbicide in maize monocultures in Hungary. In contrast to DDT, which was banned first in the world in Hungary in 1968, atrazine was benig used up to the last date possible by derogation measures upon its ban in EU in 2007. It was often detected in the US, for example, in ground water [47] together with other pesticide active ingredients (simazine, metolachlor, etc.). Diazinon insecticide was also banned in 2007. Trifluralin active ingredient is banned in Hungary since 2009; acetochlor used mainly as a herbicide in maize crops was banned in 2012. Some of these compounds are on the list of the 45 priority substances [13]. Atrazine was present at higher levels only in samples belonging to extreme point source contamination. Concentrations at these sites sometimes exceeded the values of 2000 ng/L established by the legislation as the MAC for atrazine in inland surface waters. Its levels in other water samples were far below the MRL, and upon withdrawal, its levels and occurrence frequency seem to decrease. Trifluralin, which is often detected as a water pollutant in our studies at low concentrations due to its poor water solubility, is also
listed as priority substance [13], although with no applicable MAC value.

Compared to our findings (19–70 ng/L) lower levels were reported for atrazine (<5 ng/L) from all parts of Danube in August, 2011 [48], but its metabolite desethylatrazine could be detected at levels 5–20 ng/L with maximum levels around Budapest.

Regarding chlorophenoxy acid type herbicides 2,4-D is one of the most widely used herbicides in the world and mixtures of mecoprop, dichlorprop, and MCPA are often applied. As our results indicate these compounds often occur in surface water and amounts of 2,4-D can be usually quantified (56–186 ng/L in 2011). Similar results have been reported in a study [48] conducted in August and September, 2011, with limited number of target compounds belonging to pesticides. The highest concentrations for 2,4-D were found in the area around Budapest (~50 ng/L), whereas in the Austrian-Slovakian part of the Danube and in the downstream part lower concentrations (~20 ng/L, ~10 ng/L) were measured.

Despite of the fact that glyphosate is the most frequently used herbicide in Hungary, as well as worldwide, there is little known information about its levels in the environment. Due to its fast decomposition and low detectability it is rarely measured. Regarding contamination rates and levels of glyphosate, the great contrast between sampling regimes is explained by differing agricultural locations, and, to a greater extent, catchment area characteristics, resulting in varying leaching or runoff of glyphosate to surface water. Contamination rates and levels found are strongly influenced by amounts of natural precipitation. Glyphosate contamination reported in large scale environmental water contamination studies was similar to our results. Byer et al. [27] analyzed over 700 samples in Canada using an ELISA method. Concentrations were above LOD (100 ng/L) in 33% of the samples collected in 2007, with peak values (up to 12000 ng/L) in late spring/early summer and fall. A monitoring study in Norway [49] found frequent occurrence of glyphosate and AMPA in surface water (54% of 540 surface water samples in 1995–1999). Monitoring in Catalonia, Spain, between 2007 and 2010 [26], reported a 41% contamination rate in the ground water samples analyzed. Similar findings were reported in the United States [50, 51], as well as in Canada in 2004–2005 (21% of 502 samples contained glyphosate or AMPA at very high maximum concentrations of 41 and 30 ng/mL, resp.) [52].

5. Conclusions

During this period detectable pesticide residues at low concentrations occurred in alarming proportions of the surface water samples analyzed over decades. Hardly were found samples with pesticide residues below the analytical LOD, even in natural protection or recreational areas. Among monitored pesticides, the most frequently found ingredients are mainly used in maize production. High and periodic herbicide residue levels mostly reflect current herbicide usage, while low to moderate levels of certain pesticides (e.g., trifluralin) indicate a general diffuse contamination countrywide. However, high concentrations observed at point sources were not due to agricultural pesticide application but were related to the pesticide production industry. Contamination levels in ecological fields were substantially lower than that of intensively cultivated fields. However, residues are present in organic cultivation and cause exposure due to persistent organic pollutants (POPs) in soil and due to contamination of irrigation water.

Occurrence of banned ingredients may indicate illegal pesticide use or slow decomposition in the given environmental matrix. Among often detected water pollutants some ingredients (atrazine, diazinon, and trifluralin) have been withdrawn in the meantime that can improve water quality. However, as the obtained results show, these compounds and their residues can still be detected in environmental matrices due to their slow degradation rate.

Observed pesticide residue levels in surface waters correlate with current pesticide applications and rates. The ongoing process of pesticide reevaluation in the EU resulted in re-registration of only 27% of the authorized pesticide active ingredients between 1995 and 2009 [53]. In turn, the range of available pesticides registered for crop and horticultural plant protection has substantially changed in Hungary after 2004 as the country became a full member of the EU. Among insecticides and acaricides, as well as fungicides and antimicrobials, numerous active ingredients have been withdrawn and replaced by new types (novel pyrethroid, neonicotinoid insecticides, triazole, and strobilurin fungicides). The most radical changes occurred among herbicides that represent over half of the pesticide market. In addition to several thiocarbamates (EPTC, butylate), major triazines (atrazine, cyazine, terbutryn, and prometryn) and chloroacetamides (propachlor, alachlor, propisochlor, and acetochlor) have been gradually banned. Moreover, the shrinkage in herbicide active ingredients led to the predominance of glyphosate on the herbicide market with over 30 various currently registered glyphosate-based formulations. However, on the basis of the resulting increase in environmental occurrence and exposure routes of glyphosate, as well as its recent classification in Group 2A (probably carcinogenic to humans) by the International Agency for Research on Cancer [54] glyphosate is likely to face restrictions on its use in the near future, which will, in turn, affect its levels in environmental matrices. Certain replacement (and only later banned) compounds (e.g., acetochlor) occurred as surface water contaminants. Thus, main surface water contaminants were triazines (atrazine, propisochlor), chloroacetamides (acetochlor, metolachlor), and phenoxyacetic acids (2,4-D, MCPA) during the late 1990s, followed by triazines (atrazine, prometryn, and diazinon) and chloroacetamides (acetochlor) after the turn of the millennium, while glyphosate and neonicotinoids are more frequently detected lately with the advancement of analytical techniques.

Disclaimer

This publication reflects the views only of the authors, and the European Commission cannot be held responsible for any use which may be made of the information contained therein.
Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Extraction of Water Treatment Coagulant from Locally Abundant Kaolin Clays

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Rapid industrialisation is contributing to water pollution. There is a need to identify cheaper and efficient methods of removing contaminants as the demand for clean water rises. A study is carried out to investigate the extraction of alum from locally abundant kaolin clays using sulphuric acid. Alum is a coagulant that is used for raw water treatment. The kaolin clay and alum were characterized by Fourier transformation infrared spectroscopy (FTIR). The effects of particle size, calcination temperature, calcination time, acid-kaolin clay ratio, acid concentration, leaching temperature, and leaching time on extraction efficiency were investigated. The optimum leaching conditions for the calcined kaolin clay were found to be particle size 100 \( \mu \)m, acid-kaolin clay weight ratio 6:1, acid concentration 4M, leaching temperature 100 \(^\circ\)C, and leaching time 90 min. Under optimised conditions, 66.95% (w/w) aluminum sulphate was extracted. The results showed that sulphuric acid could be used on a large scale to extract alum from kaolin clay. The extracted alum showed similar structural and physical characteristics compared with commercial alum. A dosage of 40 mg/L of the extracted alum showed effective coagulant properties with a great potential of treating raw water.

1. Introduction

Alum (aluminum sulphate) is a coagulant that is used in water treatment plants for supply of safe domestic and industrial water [1–4]. Alum is traditionally extracted from bauxite, an aluminosilicate mineral by the Bayer process [5]. During this process, the ground bauxite mineral is pressure leached with an alkali such as sodium hydroxide to obtain an aluminate solution. Pure aluminum hydroxide is then precipitated from the aluminate solution by seeding.

Another aluminosilicate mineral is kaolin which is found among different types of clays [6, 7]. Kaolin has a high Al content compared to other types of clay such as smectite, illite, and chlorite. Kaolin is a naturally hydrated aluminum silicate mineral that has a variety of colours ranging from white to red due to presence of variable amounts of iron oxide [8].

Aluminum has been extracted from kaolin clays using different mineral acids [9]. The use of hydrochloric acid for leaching alumina compared to other acids offers several advantages such as the ease of filtration of slurries, ease of iron removal, and the insolubility of titanium dioxide present in many clays [10]. The main setback of using hydrochloric acid is the severe corrosion of equipment. However, the development of corrosion resistant rubbers and plastics has solved this problem to a large extent. Both hydrochloric and sulphuric acids extract approximately the same amounts of alumina from same quantities of clay [11, 12]. The effects of reaction parameters on alumina extraction from kaolin clay have been reported in the literature [13–16].

Other aluminosilicate forms such as coal fly ash can be utilised for the extraction of alumina. Alkali treatment on desilicated coal fly ash was reported to extract 89-90% alumina [17, 18]. Wu et al. [19] reported 82.4% \( \text{Al}_2(\text{SO}_4)_3 \) extraction efficiency using sulphuric acid. Both alkali and acid dissolution processes on fly ash suffer high energy consumptions drawbacks that are not economical at industrial scales [17].
This study focuses on determining the feasibility of leaching aluminum from locally abundant Zimbabwean kaolin clays using sulphuric acid. It aims at optimizing the conditions for acid extraction of low iron content alumina. The extracted alum would be tested for industrial wastewater treatment. Currently the country imports aluminum sulphate which is costly. There is need to identify local sources of the coagulant to reduce or eliminate import costs.

2. Materials and Methods

2.1. Sample Collection. Kaolin samples were collected from Indiva Siding quarry which is located 38 km east of Gweru, Zimbabwe. The random point sampling method was applied for sample collection using a metal scoop and a metal container. Four samples were collected into 5 kg metal containers. A composite sample was then prepared by uniformly mixing the four samples using a shovel. Cone and quartering method was used to obtain smaller fractions of the composite sample. The samples were thoroughly mixed and collected into labelled plastic bags [17].

2.2. Sample Preparation and Activation. The clay samples were ground using a ball mill to particle sizes in the range 50–200 μm. The ground clay samples (20 g) of different particle sizes (50–200 μm) were put in vitreous crucible and subjected to high temperatures in a muffle furnace (carbolite) for thermal activation [17]. The calcination temperature was varied between 500 and 900°C for one hour.

2.3. Kaolin Characterisation. The elemental composition of powdered kaolin was determined using XRF (Panalytical Zetium) according to a previously reported procedure [20, 21]. Kaolin samples were pressed into pellets using boric acid before XRF analyses. The kaolin samples (1 g) were subjected to a temperature of 900°C in a muffle furnace to determine the loss on ignition (LOI).

KBr pressed pellets solid kaolin samples were analysed using FTIR spectrophotometer (Nicolet 6700).

2.4. Acid Leaching. All leaching experiments were performed in triplicate. The calcined kaolin samples (5 g) of different sizes (50–200 μm) were leached using wide ranging concentrations of H₂SO₄ (1–5 M) over varying durations (10–180 min), at different temperatures (25–100°C) and various acid/clay ratios (4:1 to 7:1) w/w under reflux and constant stirring (120 rpm). A separating funnel was used to slowly add 100 mL of ethanol into the acid leach liquor under continuous stirring to precipitate aluminum sulphate. After leaching, the mixture was filtered using Whatman number 1 filter paper. The residue was washed thrice with 30 mL aliquots of deionised water. The filtrate and washings were separately analysed for aluminium ion content using Flame Atomic Absorption Spectrometer (Shimadzu AA-6800).

2.5. Coagulation Tests. Four jar test beakers were filled with 1 L industrial wastewater. Different dosages (10–40 ppm) of the extracted and commercial aluminum sulphate were separately added to the wastewater [1]. The jars were continuously stirred for 10 minutes before allowing water to settle for 15 minutes. The turbidity, conductivity, total dissolved solids, and pH of the settled water were determined. Coagulations tests were repeated.

3. Results and Discussion

3.1. Characterisation of Kaolin by XRF. The chemical composition of kaolin is shown in Table I. Aluminum oxide and silicon dioxide were the major components of kaolin. The percentage composition of alumina (20.34%) was lower than values reported in previous studies. Kaolin samples containing 29.4% aluminum oxide were reported to achieve an extraction of 32% Al₂(SO₄)₃ [19]. Numluk and Chaisena [20] used kaolin samples containing 22.7% Al₂O₃. High silica content (58.02%) formed greater part of the insoluble residue after acid treatment. Numluk and Chaisena [20] reported samples containing 66.30% silica content. Oxides of iron, calcium, magnesium, manganese, sodium, potassium, and titanium had compositions below 2.5%.

Loss on ignition (LOI) value of 10.95% was recorded. The value is attributed to bound hydroxyl ions in calcined kaolin [16]. Aderemi et al. [22] reported a loss on ignition of 14.15% from a kaolin sample calcined at 750°C for 2 h. These values compare closely to the theoretically expected value of 13.96% (w/w) attributed to kaolin hydroxyl composition [7].

3.2. FTIR Analysis of Kaolin before and after Acid Treatment. The FTIR spectra of kaolin before and after acid extraction are shown in Figures 1 and 2. The FTIR spectrum of kaolin before acid treatment showed a band at 3460 cm⁻¹ which is assigned to inner hydroxyl stretch. This band, attributed to water physisorbed on kaolin surface, disappeared after acid treatment. The peak was a result of bonding between protons and oxygen atoms coordinated to aluminum ions in the octahedral structural layer [6]. The band observed at 465 cm⁻¹ was attributed to Si–O–Si bond deformation.

| Compound | Composition (wt.%) |
|----------|--------------------|
| Al₂O₃    | 20.34              |
| CaO      | 2.29               |
| Fe₂O₃    | 1.35               |
| K₂O      | 2.02               |
| MgO      | 1.99               |
| SiO₂     | 58.02              |
| TiO₂     | 2.05               |
| MnO₂      | 0.01               |
| Na₂O     | 0.13               |
| K₂O      | 0.17               |
| SO₃      | 0.23               |
| P₂O₅     | 0.45               |
| LOI      | 10.95              |

Table I: Chemical composition of kaolin.
The bands at 3735, 3663, and 3629 cm\(^{-1}\) observed in acid treated kaolin could be due to OH stretching of inner surface hydroxyl groups in the Al–OH in the octahedral layers of kaolin [23]. The peak intensity of 913 cm\(^{-1}\) band decreased after acid leaching showing weakening of the kaolin structure due to neutralisation of the hydroxyl groups and leaching of Al\(^{3+}\) ions. A new band appeared at 911 cm\(^{-1}\) after acid treatment which could be attributed to OH deformation of inner hydroxyl groups in the bonding of Al–Al–OH octahedral sheet [24]. The 489 and 463 cm\(^{-1}\) bands were attributed to Si–O–Si deformations. The band intensity at 489 cm\(^{-1}\) increased after acid leaching.

3.3. Effect of Calcination Temperature. Calcination temperature is a very important parameter when investigating the extraction of aluminum from kaolin clays. Calcination thermally activates the kaolin to a more reactive form [16]. The effect of calcination temperature on the extraction of aluminum sulphate is shown in Figure 3. The quantity of alumina extracted increased with increasing calcination temperature from 22.9% at 500°C to a maximum of 28.8% at 800°C. Above 800°C, the quantity of alumina extracted decreased. Thermal treatment leads to loss of water molecules within the kaolin clay structure. Above 800°C, total dehydration of kaolin clay occurs resulting in disorderly phase transformation and disruption of kaolin structure into metakaolin amorphous solid that is less prone to acid attack [16, 23]. Similar work done by Ajemba and Onukwuli [25] showed that calcined clays at 750°C exhibited more alumina dissolution rates compared to those at lower temperatures. Active extraction was observed to begin at 500–600°C. Numluk and Chaisena [20] reported that the percentage of alumina extracted increased with increasing calcination temperature in the range 550–850°C and decreased sharply above 850°C. Numluk and Chaisena [20] managed to achieve high alumina dissolution of 52.2% at a calcination temperature of 750°C. Studies carried out by Aderemi et al. [22] on kaolin reported maximum alumina extraction at 800°C.

3.4. Effect of Calcination Time. Figure 4 shows the effect of calcination time on the yield of aluminum sulphate. The percentage of alum extracted increased up to 60 minutes. Beyond 60 minutes the amount of alum extracted leveled off at 30%. Low calcination times could result in insufficient thermal treatment of the kaolin sample. Increasing time ensures that kaolin samples are adequately exposed to calcination. Similar
results were reported by Al-Zahrani and Abdul-Majid [26] giving maximum of 62.94% alumina yields.

3.5. Effect of Particle Size. The particle size of kaolin has an influence on the extraction efficiency of aluminum sulphate. Figure 5 shows the effect of kaolin particle size on the quantity of alum extracted. The quantity of aluminum sulphate extracted increased with increasing particle size before leveling off at 90 μm. The maximum extraction efficiency (25.7%) was observed between 90 and 150 μm. Above 150 μm particle size, a decrease in the quantity of aluminum sulphate extracted was observed. The decline could be attributed to decreased clay surface area exposed for acid attack. Smaller particle sizes exhibit a larger surface area. The work done by Al-Zahrani and Abdul-Majid [26] concluded that a particle size of 149 μm resulted in maximum aluminum sulphate extraction (32%). Particle sizes of 100 μm were recommended in this work for further leaching experiments.

3.6. Effect of Leaching Temperature. Conventional aluminum sulphate extraction method using heated water depends on the bath temperature. Figure 6 shows the effect of water bath temperature on aluminum sulphate extraction. Increasing leaching temperature results in increased yield of aluminum sulphate. An increase in leaching temperature increases the kinetic energy of the kaolin-acid solution resulting in increased collision frequency. The highest degree of aluminum sulphate extraction was observed at boiling temperature (100°C). A study done in comparing conventional and microwave heating methods showed that the latter produced higher extraction yields due to attainment of elevated leaching temperature [27]. Other studies concluded that kaolin leaching requires high temperatures [25]. Another study investigating the effect of leaching temperature also showed that the highest yield of aluminum sulphate was obtained at boiling temperature [26].

3.7. Effect of Leaching Time. The effect of leaching time on the extraction process is shown in Figure 7. The yield of alum increased with increasing leaching time. The percentage yield of alum leveled off at 45% after 90 minutes. Prolonged leaching time allowed ample time of interaction between the acid and kaolin particles. Al-Zahrani and Abdul-Majid [26] reported that the percentage of aluminum sulphate extracted attained a constant value after 1 h at all leaching temperatures. Numluk and Chaisena [20] attained maximum alum extraction after 120 minutes.

3.8. Effect of Acid to Clay Weight Ratio. The effect of acid to kaolin clay ratio on aluminum sulphate extraction is illustrated in Figure 8. The acid-solid ratio plays a crucial role in the extraction of aluminum sulphate from kaolin. This parameter indicates the amount of acid needed to be in contact with solid for optimum extraction. There was an increase in percentage of aluminum sulphate extracted as the acid-solid ratio increased. The maximum yield of alum (33.1%) was achieved at an acid-solid ratio of 6:1. Above 6:1 acid-clay ratio the alum yield remained constant probably due to a saturation of available clay sites with hydrogen ions. Increasing acid-clay ratio increased the amount of H\(^{+}\) ions that interact with kaolin particles until saturation was attained. Previous studies done by Al-Zahrani and Abdul-Majid [26] showed that increasing the ratio from 4:1 to 10:1 increased quantity of aluminum sulphate extracted. Similar studies revealed that the percentage of extraction of
Table 2: Optimised sulphuric acid extraction conditions for alum from kaolin clay.

| Particle size (μm) | Acid conc. (M) | Acid:kaolin ratio (w/w) | Leaching Temperature (°C) | Leaching Time (min) | Calcination Temperature (°C) | Calcination Time (min) | Extraction (wt.%) |
|--------------------|---------------|-------------------------|---------------------------|---------------------|-----------------------------|------------------------|------------------|
|                    |               | 6:1                     | 100                       | 90                  | 800                         | 60                     | 66.95            |

alumina increased by increasing the acid-clay ratio reaching a maximum value of 30.16% and 65.8% for uncalcined and calcined samples, respectively, at the ratio of 10:1 [28].

3.9. Effect of Acid Concentration. Figure 9 shows the effect of acid concentration on the extraction of aluminum sulphate. The degree of extraction increased with increase in acid concentration. The percentage yield of alum increased from 24.9% at 0.5 M to 31.1% at 4 M H₂SO₄. A decrease in aluminum sulphate extracted was observed above 4 M H₂SO₄. An increase in acid strength increases the diffusion of H⁺ ions into the octahedral layer of kaolin resulting in increased leaching of aluminum ions. At very high acid concentration the structure of sample collapses and the Al³⁺ ions block the diffusion of H⁺ [26]. Numluk and Chaisena [20] obtained a high degree of aluminum sulphate extraction of 39.0%.

3.10. Optimum Conditions for Aluminum Sulphate Extraction. Table 2 shows the alum yields obtained under optimum extraction conditions. Leaching kaolin clay under these conditions yielded 66.95% alum. The yield compares favourably with previously reported extractions. Al-Zahrani and Abdul-Majid [26] reported 63% alum extraction efficiency from kaolin using hydrochloric acid.

3.11. Comparative and Physical Properties of Extracted and Commercial Alum. The melting points, pH, and colour of the commercial and extracted alumina are compared closely as illustrated in Table 3.

3.12. Coagulation Tests. The coagulation tests using locally extracted (I) and commercial (C) alum on industrial wastewater are shown in Table 4. The wastewater had a turbidity of 16.7 Nephelometric Turbidity Units (NTU) depicting high amounts of organic matter, mud, silt, and other inorganic precipitates that gave it a cloudy appearance. Commercial alum was more effective in removing turbidity than the extracted coagulant. A coagulant dose of 40 mg L⁻¹ of the local alum resulted in reduction of turbidity to 6.4 NTU whereas only 25 mg L⁻¹ of the commercial alum was required to obtain WHO recommended turbidity of 0–5 NTU [3]. The conductivity of the water after coagulation increased due to additional ions from the coagulants. There were no significant differences in conductivity changes using both alums. The pH of the clarified water decreased after alum treatment using both alums which is in agreement with previous studies due to partial hydrolysis of aluminum sulphate [1, 28]. The two coagulants exhibited similar tendencies in removing natural organic matter as reflected by similar changes in total organic content (TOC). The in-house alum therefore exhibits good coagulation tendencies and compares favourably with the commercial alum with the exception of its effectiveness in removing turbidity.

4. Conclusion

This study demonstrated that aluminum sulphate can be efficiently extracted from local kaolin. The extracted alum has shown great potential in removing pollutants from industrial
wastewaters. Approximately 67% of alum was extracted from kaolin clay calcined at 800°C for 1 h. The optimum leaching conditions were leaching 100 μm clay particle sizes with 4 M sulphuric acid at 100°C for 1.5 h using acid to clay ratio of 6:1. The coagulant produced demonstrated huge potential for treating industrial wastewater. The coagulant however fell short in lowering turbidity to recommended levels making the treated water unsuitable for human consumption. Extracting alum from local kaolin at industrial scale has great potential in reducing coagulant import costs. Further studies aimed at converting the extracted alum to more effective polyaluminum chlorides coagulant are recommended.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Three-Dimensional Modeling of Hydrodynamics and Biokinetics in EGSB Reactor

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A three-dimensional model integrating computational fluid dynamics (CFD) and biokinetics was established to model an expanded granular sludge bed (EGSB) reactor in this study. The EGSB reactor treating synthesized municipal wastewater was operated at ambient temperature. The model provided satisfactory modeling results regarding hydrodynamics and biokinetics. The model shows that influent distribution was evenly distributed. In addition, butyrate and propionate degradation rates linearly decreased along the flow direction in the reactor. However, acetate degradation rate increased first and decreased later. VFA degradation rate distributions were different at each reactor cross section. VFA degradation rates near reactor wall were lower than VFA degradation rates at reactor axis. Moreover, a pulse high influent COD concentration had a tiny impact on effluent quality, which indicates that the reactor was stable while treating synthesized wastewater at adopted conditions.

1. Introduction

Expanded granular sludge bed (EGSB) is a granular sludge-based wastewater treatment technology and has been widely applied in high strength wastewater treatment. In addition, many researches regarding low strength wastewater treatment, that is, municipal wastewater treatment, have been reported [1–3].

Vertical slim columns are applied as structures of EGSB reactors. However, a staged reactor structure resulted in a more efficient reactor [4], which indicates that there is a need for the optimization of EGSB reactor structure. The optimization requires insight of hydrodynamics as well as biokinetics in EGSB reactors. The hydrodynamics and biokinetics in upflow anaerobic sludge blanket (UASB) were analyzed, which resulted in a model integrating hydrodynamics and biokinetics [5]. However, the integrated model was too complex and parameterized.

Alternatively, by the application of computational fluid dynamics (CFD), the hydrodynamics as well as mass transfer in EGSB reactors could be well obtained. Up to now, a few works applying two-dimensional (2D) CFD models have been reported [6–9]. However, if a flow field is too complex to be simplified as a 2D model, a three-dimensional (3D) CFD model is required. Considering the complexity of flow near the inlet of an EGSB reactor, it could be better to apply a 3D CFD model. Regarding 3D CFD models, a few reports about continual stirred flow reactors and aerobic bioreactors as well as anaerobic digesters were reported [10–13]. Nevertheless, reports regarding 3D CFD models about EGSB reactors are still not available.

Moreover, while hydrodynamics and biokinetic are needed to be integrated, it is considered that a 3D hydrodynamic model is better to be applied than applying a 2D CFD model. That is because real mass transfer conditions can be better modelled. After extensive 3D CFD modelling work regarding anaerobic digesters having been done, flow and biokinetics in anaerobic digesters are well known [7, 14–16]. Nevertheless, these researches were not related to EGSB reactors.

Influent distribution is considered playing an important role in reactor operations and especially essential at large scales. If influent distribution is required to be included in a CFD model, a 3D rather than a 2D CFD model is required.
2. Methods and Materials

2.1. EGSB Operation. Peristalsis pumps (Jihpump BT-100EA, China) were applied for providing influent and backflow to a lab scale EGSB reactor. Hydraulic retention time was 2.5 hours and upflow velocity was maintained at 5 m/h. Inoculum sludge was taken from an internal circulation reactor treating pulping wastewater (Chongqing, China). The EGSB reactor was fed with synthetic wastewater prepared by volatile fatty acids (VFA). The chemical oxygen demand (COD) ratio of acetate:propionate:butyrate was 2:1:1 while total COD concentration of the influent was between 350 and 430 mg/L. Ammonium concentration and phosphate concentration were 30 mg/L and 4 mg/L, respectively. NaHCO₃ was applied to maintain pH between 6.8 and 7.5. During the operation of the reactor, volatile suspended solid (VSS) was measured by standard method [17]. VFA were measured by high pressured liquid chromatography (DIONEX Ultimate 3000 HPLC, USA). The reactor was operated at 26°C.

2.2. EGSB Modelling

2.2.1. Included Biokinetics. After operating the reactor for 75 days, effluent COD and the height of sludge bed were stable. VSS concentration decreased along the flow direction in the reactor and is given by

\[ X = (88.32 - 1.92 \times h) \times 1000, \]  

where \( X \) is concentration of VSS, mg/L; \( h \) is reactor height, 0~46 cm.

VFA were degraded in the sludge bed. Therefore, three bioprocesses shown in Tables 1 and 2 were included in the integrated model. VSS concentrations shown in (1) were included and were constant in the integrated model. Therefore, biomass decay and growth were not necessary to be included in the applied biokinetics.

2.2.2. Model Geometry. In the EGSB reactor, a sedimentation zone (free flow zone) and a sludge bed right under the sedimentation zone were observed. COD was degraded in the sludge bed and almost no COD degradation took place in the sedimentation zone. In this model, the sludge bed was treated as a porous bed and no reaction occurred in the sedimentation zone. Two different modeling domains, that required different model strategies, are shown in Figure 1.

2.2.3. Meshing. The modelling domains were meshed in COMSOL Multiphysics (version 4.3 a) that was the model platform of this study. Average element quality was 0.52 cm while the minimum element quality was 0.0058 cm. The meshing was sufficiently fine and did not have an impact on modelling results.

2.2.4. Hydrodynamics and Mass Transport Modeling. As shown in (1), sludge concentrations at different locations in the EGSB reactor were different, which is different from well mixed anaerobic digesters and aerobic bioreactors [10–13]. Sludge concentration has a big impact on sludge viscosity,
which made sludge a non-Newtonian liquid [18]. However, water in the free flow zone was a Newtonian liquid. Therefore, influent flowed into a non-Newtonian phase and a Newtonian liquid consequently, which resulted in a modeling difficulty. Nevertheless, sludge bed can be treated as a porous bed while influent flowed in space between sludge particles. By this kind of treatment, it was not the sludge viscosity but sludge bed porosity and permeability that impacted sludge flow in the free flow zone and then into the sludge bed.

The porosity and permeability of sludge bed were calculated by (2) and (3), respectively:

$$\varepsilon_p = \frac{\rho_g - \rho_i}{\rho_g - \rho_w},$$  \hspace{1cm} (2)

$$\kappa = \frac{\nu\Delta x}{\Delta p},$$  \hspace{1cm} (3)

where $\varepsilon_p$ is porosity; $\rho_g$ is wet granular density, kg/m$^3$; $\rho_i$ is sludge bed density, kg/m$^3$; $\rho_w$ is water density, kg/m$^3$; $\kappa$ is permeability, m$^2$; $\nu$ is wastewater superficial velocity, m/s; $\Delta x$ is sludge bed height, m; $\Delta p$ is pressure different across sludge bed height, Pa; $\mu$ is water viscosity, Pa/s.

In the sedimentation zone, limited sludge existed and the zone was a free flow zone. Navier-Stokes equations ((4)-(5)) were applied to model the flow in the free flow zone. As for the mass convection and diffusion, (6) was applied in the free flow zone and sludge bed. Sludge bed was treated as a porous bed in the model. Brinkman equations ((7)-(8)) were applied to model hydraulic dynamics in the sludge bed.

Continuity in the free flow zone is as follows:

$$\frac{\partial \rho}{\partial t} + \nabla (\rho \mathbf{u}) = 0.$$  \hspace{1cm} (4)

Momentum in the free flow zone is as follows:

$$\frac{\rho}{\varepsilon_p} \frac{\partial \mathbf{u}}{\partial t} + \nabla \rho \mathbf{u} = -\nabla p + \nabla \left\{ \mu \left( \mathbf{u} + \left( \nabla \mathbf{u} \right)^T \right) - \frac{2}{3} \mu \left( \nabla \mathbf{u} \right) I \right\} + \mathbf{F},$$  \hspace{1cm} (5)

Mass convection and diffusion are as follows:

$$\frac{\partial c}{\partial t} + \mathbf{u} \nabla c = D \nabla^2 c + R.$$  \hspace{1cm} (6)

Continuity in the sludge bed is as follows:

$$\frac{\delta (\varepsilon_p \rho)}{\partial t} + \nabla (\rho \mathbf{u}) = Q_{br}.$$  \hspace{1cm} (7)

Momentum in the sludge bed is as follows:

$$\frac{\rho}{\varepsilon_p} \frac{\partial \mathbf{u}}{\partial t} + \nabla \rho \mathbf{u} = -\nabla p + \nabla \left\{ \frac{1}{\varepsilon_p} \left[ \mu \left( \mathbf{u} + \left( \nabla \mathbf{u} \right)^T \right) - \frac{2}{3} \mu \left( \nabla \mathbf{u} \right) I \right] \right\} - \frac{\mu}{\kappa} \frac{Q_{br}}{\varepsilon_p^2} \mathbf{u} + \mathbf{F},$$  \hspace{1cm} (8)

where $c$ is concentration of species, mol/m$^3$; $D$ is diffusion coefficient, m$^2$/s; $R$ is reaction rate expression for the species, mol/(m$^3$⋅s); $\mathbf{u}$ is the velocity vector, m/s; $\rho$ is density of the fluid, kg/m$^3$; $p$ is pressure, Pa; $Q_{br}$ is a mass source or mass sink, kg/(m$^3$⋅s); $\mathbf{F}$ is volume force, N/m$^3$; $I$ is unit matrix.

2.2.5. Boundary Conditions. Inlet boundary condition was velocity inlet, while pressure and no viscous stress boundary condition was applied to the outlet. The latter boundary condition corresponds to (9). At the outlet, convection and migration were the governing mass transport mechanisms and diffusion transport was ignored:

$$\left[ \mu \left( \nabla \mathbf{u} + \left( \nabla \mathbf{u} \right)^T \right) \right] \cdot \mathbf{n} = 0,$$  \hspace{1cm} (9)

where $\mathbf{n}$ is a unit vector.

2.2.6. Solver and Converge Conditions. Stationary solver was applied to solve the integrated model. The solution converged while residual errors for pressure, momentum, and species concentrations were below 0.001.

2.2.7. Tracer Experiments. The hydrodynamics and convection-diffusion of mass in the model were verified by applying CaCl$_2$ as a tracer. CaCl$_2$ was injected into the reactor.
while an influent distribution plate was fixed at the bottom of the reactor and the reactor was filled with clean water.

While sludge presented in the reactor, tracer experiment was not performed because potential adsorption and precipitation of Ca\(^{2+}\) might result in loss of the tracer. LiCl is generally applied as a tracer in bioreactors. However, because analysis of Li\(^+\) could not be performed while this research was carried out, tracer experiments were not performed while sludge presented in the reactor. Ca\(^{2+}\) concentrations were measured by standard method [17].

3. Results and Discussions

Figure 2 shows the Ca\(^{2+}\) concentrations obtained at the outlet of the EGSB reactor during the tracer experiment. Simulated Ca\(^{2+}\) concentrations matched experimental Ca\(^{2+}\) concentrations well, which indicates that the applied equations ((4)–(6), (9)) could be applied to model hydrodynamics and mass transfer in the reactor where no sludge presented.

VFA concentrations at sampling points located at reactor wall were shown in Figure 3. Although the influent total VFA concentrations were between 350 and 430 mg/L, the applied backflow largely diluted the influent VFA concentrations, which resulted in low real reactor influent VFA concentrations. Because propionate and butyrate concentrations at every sampling point (shown in Figure 3) were below detection limits of the two fatty acids (4 mg/L), propionate and butyrate concentrations could not be measured. In addition, almost all VFA were degraded in the sludge bed because no VFA could be detected at the outlet of the EGSB reactor.

Figure 3 shows that measured and simulated acetate concentrations matched well, which indicates that applied hydrodynamics and mass transfer as well as biokinetic models were acceptable. The majority of propionate and butyrate were degraded at the bottom of the reactor, while acetate was quickly removed in the sludge bed.

Moreover, Figures 2 and 3 indicate that hydrodynamics and mass transfer in the EGSB reactor could be modeled by ((4)–(9)).

Hydrodynamics is important for bioreactors, as shortcut flow can result in low reactor efficiency. As for high rate anaerobic bioreactors, shortcut flow indicates that local organic load can be much higher than an expected value and consequently result in a poor reactor efficiency. Therefore, influent distribution at the inlets of high rate anaerobic reactors is important for overall reactor efficiency [19].

Traditionally, modeling of an aerobic wastewater bioreactor relies on a hydrodynamic model that treats the reactor as a combination of a number of ideal reactors (CSTRs or plug flow reactors). Then, by the application of activated sludge models to each ideal reactor, effluent quality of a modeled reactor can be obtained. However, the construction of the hydrodynamic model is difficult and relies on experience [20]. Hydrodynamic models regarding anaerobic granular based reactors are much more complex than those regarding aerobic bioreactors [21], which makes modeling anaerobic bioreactors difficult. Alternatively, hydrodynamics can be modeled by a few equations ((4)-(5), (7)-(8)), which provides an accurate hydrodynamic modeling.

Figure 4 shows the distribution of influent at the bottom of the reactor. While a distribution plate was not applied (Figure 4(a)), influent could directly flow upwards with relative high velocity, which indicates that shortcut was incurred and reactor space was not fully utilized. While a distribution plate was applied (Figure 4(b)), though influent velocity near the middle of the distribution plate was higher than the surrounding liquid velocity, the influent was much more uniformly distributed in the entire reactor cross section. The sludge bed was a porous bed and provided flow resistance to the influent, which resulted in further improvement in influent distribution (Figure 4(c)). After flowing through the distribution plate, influent velocity immediately approached the required velocity, which indicates that a good influent distribution design was achieved.
Up to now, relationships between operation parameters such as solid retention time, effluent quality, sludge activity, and microbial ecology variation have been well understood [1, 22–25]. However, many aspects such as reaction rates at each point in a reactor are hard to know as obtaining relative parameters at each point is not practical.

Figure 5 shows the VFA degradation rates in the sludge bed. The reaction rates of propionate and butyrate decreased along the flow direction in the reactor, which was different from that of acetate. The reaction rate of acetate increased and then decreased afterwards. That should derive from the conversion of butyrate and propionate into acetate, which resulted in a reaction peak. The distribution of reaction rates indicates that, due to the well influent distribution, a plug flow was well created in the reactor, which was beneficial for VFA degradation. Figure 5 also shows that VFA degradation rate distributions were different at each reactor cross section. VFA degradation rates near the reactor wall were lower than VFA degradation rates at the reactor axis, which is clearly shown in Figure 6. The maximum reaction differences were in the range of 0.2–0.4 mg/L·s. Nevertheless, the difference gradually decreased along the flow direction.

Reactor influents generally are provided by pumping that offers stable influent flow; therefore organic load fluctuation mainly comes from influent COD concentration variation, which results in effluent quality variation. When EGSB reactors are applied to municipal wastewater treatment, stability of effluent quality is interesting since influent COD level always fluctuates. The stability of the reactor was tested by providing a pulse COD load to the reactor in the model. The duration of the pulse COD load was 300 seconds. Influent COD increased from 300 mg/L to 600 mg/L, while influent...
Figure 5: Distribution of VFA degradation rates in the EGSB reactor. From left to right: acetate, propionate, and butyrate (mg/L·s).

Figure 6: VFA degradation rates along sludge bed height at reactor axis (axis) and near reactor wall (wall) (mg/L·s).

Flow velocity was constant. Figure 7 shows that the pulse COD load started and finished at 500 seconds and 800 seconds, respectively. The response of the pulse COD load showed that effluent VFA concentrations increased as the result of the influent COD pulse. However, VFA concentrations were still below the VFA detection limits. The butyrate concentration was quite low and Figure 7 can almost not show it. The stability test indicates that no matter how sharply COD load fluctuates, if COD load is always between 300 mg/L and 600 mg/L, the EGSB reactor could stably maintain VFA concentrations below the detection limits.

4. Conclusions

A 3D CFD model was established, which included the effect of influent distribution in reactor hydrodynamics. In the model, the sludge bed in the reactor was treated as a porous bed, which was different from other publications that treated the sludge bed as a single phase that is different from water phase. Furthermore, the CFD model integrated biokinetics together, which could provide reactor operation details at different points in the reactor. Satisfactory modeling results regarding hydrodynamics and biokinetics were obtained. Based on this study, three conclusions can be obtained:

1. The designed distribution plate could efficiently distribute influent at the bottom of the reactor, and the presence of sludge bed improved the influent distribution.

2. The pulse increase of influent COD concentration from 300 mg/L to 600 mg/L had tiny impact on effluent quality, which showed good stability of the EGSB reactor.

3. The degradation rate of acetate increased and then decreased along the flow direction in the reactor, while the reaction rates of propionate and butyrate decreased along the flow direction. VFA degradation rate distributions were different at each reactor cross section. VFA degradation rates near the reactor wall were lower than VFA degradation rates at the reactor axis.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.
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