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Efficacy of Continuous Flow Reactors for Biological Treatment of 1,4-Dioxane Contaminated Textile Wastewater Using a Mixed Culture

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Abstract: The goal of this study was to evaluate the biodegradation of 1,4–dioxane using a mixed biological culture grown in textile wastewater sludge with 1,4–dioxane as the sole carbon source. The conditions for the long-term evaluation of 1,4–dioxane degradation were determined and optimized by batch scale analysis. Moreover, Monod's model was used to determine the biomass decay rate and unknown parameters. The soluble chemical oxygen demand (sCOD) was used to determine the concentration of 1,4–dioxane in the batch test, and gas chromatography/mass spectrometry (GC/MS) was used to measure the concentrations via long-term wastewater analysis. Two types of reactors (continuous stirred reactor (CSTR) and plug flow reactor (PFR)) for the treatment of 1,4–dioxane from textile wastewater were operated for more than 120 days under optimized conditions. These used the mixed microbial culture grown in textile wastewater sludge and 1,4–dioxane as the sole carbon source. The results indicated efficient degradation of 1,4–dioxane by the mixed culture in the presence of a competitive inhibitor, with an increase in degradation time from 13.37 h to 55 h. A specific substrate utilization rate of 0.0096 mg 1,4–dioxane/mg MLVSS/h was observed at a hydraulic retention time of 20 h for 20 days of operation in a biomass concentration of 3000 mg/L produced by the mixed microbial culturing process. In the long-term analysis, effluent concentrations of 3 mg/L and <1 mg/L of 1,4–dioxane were observed for CSTR and PFR, respectively. The higher removal efficacy of PFR was due to the production of more MLVSS at 4000 mg/L compared to the outcome of 3000 mg/L in CSTR in a competitive environment.

Keywords: 1,4–dioxane; biodegradation; mixed microbial culture; continuous stirred reactor (CSTR); plug flow reactor (PFR)

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1. Introduction

Municipal wastewater effluent (MSW) contains various hazardous and toxic pathogens that must be removed before discharge and reclamation, with 1,4–dioxane among them [1–5]. Despite being widely used as a solvent in various industries, including the pharmaceutical, textile, cotton and adhesives industries [6,7], environmental and public health concerns about 1,4–dioxane are growing due to its carcinogenicity in humans and it being listed as a priority hazardous pollutant [8–11]. Moreover, limited regulations pertaining to the release

of 1,4–dioxane enable its wide distribution in the environment [12,13]. The US regulates 1,4–dioxane discharges as hazardous when used as an industrial solvent; however, its disposal is not regulated for other applications [14]. Therefore, it is necessary to eliminate 1,4–dioxane from water before it comes into contact with humans.

In recent decades, significant improvements have been made to overcome chemical and microbial threats [15] to water supplies, mainly through a series of treatment strategies such as membrane filtration [16–22], the advanced oxidation process (AOP) [23,24] and bioremediation. Contaminants, such as 1,4–dioxane, considered a contaminant of emerging concern, can be treated via reverse osmosis in combination with AOP to comply with water quality guidelines that stipulate only 1 µg/L [1]. However, while UV-based AOP successfully removes up to 99% of 1,4–dioxane, it adds additional cost to the treatment, making it exceptionally expensive [15,25]. A similar issue was observed for distillation, as this thermal process operates at a high temperature. In addition, traditional disinfection processes cannot be adopted, as they remove 1,4–dioxane but produce byproducts which are more hazardous than 1,4–dioxane itself [7,26]. AOP and bioremediation are generally used for the treatment of cyclic ethers. Moreover, AOP by ozonation was found to be ineffective for the degradation of 1,4–dioxane, as the hydroxyl radicals (OH) produced through the AOP mechanism, which are responsible for degradation and ozonation, could not be produced in sufficient amounts [24,27]. In the literature, different combinations, including H_2O_2 and UV-based AOP, have been used to treat 1,4–dioxane. It has been found that H_2O_2 at a dose of 3–5 mg/L and UV radiation with a wavelength of 254 nm can effectively degrade 1,4–dioxane [28,29]. Helen et al. [7] demonstrated that in-line ozonation was more effective within the range of a basic medium (pH > 9), reducing 90% of the chemical oxygen demand and completely removing 1,4-dioxane due to the formation of ethylene glycol as an intermediate [7]. However, constraints related to practical applications involved the pretreatment of influent water [30], as the presence of other pollutants reduces the efficiency of 1,4–dioxane and produces other byproducts by scavenging radicals [6]. Moreover, the removal efficacy of AOP treatment is also limited due to the need for continual dosing, the provision of high energy, and the need for certain chemical conditions.

In the literature, the bioremediation of 1,4–dioxane has shown that it is a non–biodegradable pollutant [31–33] and it cannot be removed effectively in the presence of other organic pollutants with a microbial treatment [34]. However, optimization of the process and other recent advancements have enabled various types of microbial growth that can effectively degrade 1,4–dioxane depending upon the use of a suitable carbon source and the microbial community structure [35,36]. Generally, a single-strain microorganism is grown on tetrahydrofuran (THF), which is used to oxidize the 1,4–dioxane [37,38]. However, 1,4–dioxane catabolism initiated by monooxygenases and a THF degrader is ineffective if used for degradation [30,39]. Anaerobic bioremediation using Fe(III)-reducing bacterium sludge was used to degrade 1,4–dioxane, and its efficacy was increased by an up-flow biological filter [33]. However, the removal efficiency decreases rapidly with an increase in the ratio of CO to 1,4–dioxane. A bio-augmented approach was also considered to be effective for the degradation and removal of 1,4–dioxane by growing a bacterial strain on an activated carbon adsorbent [40]. In most cases, certain metals and chlorinated solvents are considered limiting factors due to inhibition [41,42]. In the literature, less attention has been paid to the growth of a mixed culture and microorganisms considering 1,4–dioxane as the sole carbon source [4,43]. Moreover, most of these studies have been conducted on the batch scale for the bioremediation of 1,4–dioxane in a controlled environment. Relatively few studies have analyzed bacterial strains in the long term [7,31]. The treatment of wastewater in real-world applications can be performed in a continuous manner, using aeration tanks and clarifiers, with an emphasis on the cultivation of mature bacteriological species to use in the contaminated treatment. An example of this is the use of an aerobic granular sludge (AGS) system in a sequential batch reactor (SBR) as a continuous flow reactor for the treatment of wastewater. Moreover, the treatment configurations depend on the hydraulic shear force and selective pressure [44,45]. Similarly, the application of a continuous biological reactor

to treat 1,4-dioxane also depends on various factors, including hydraulic retention time (HRT), microbial growth species, mixed liquid volatile suspended solids (MLVSS), influent concentrations and impacts of the inhibitor. Moreover, efficient degradation of 1,4–dioxane also depends on the type of treatment configuration (such as CSTR and PFR, in this study). In line with similar methodologies, here we grow mixed microbial cultures from sludge sourced from the textile industry, optimized by a batch analysis, and analyzed long-term for 120 days under various conditions and with two types of reactors.

The goal of this study is to analyze the degradation of 1,4–dioxane discharged from textile industry wastewater using mixed biological culture seeding under the operation of a continuous stirred reactor (CSTR) and a plug flow reactor (PFR) for 120 days. Moreover, Monod models were used to compare the predicated and actual levels of biological degradation of 1,4–dioxane. In addition, bench-scale studies were conducted to standardize the parameters for evaluation. The impact of variations in hydraulic retention time (HRT) was also analyzed to find the optimum HRT and optimum biomass concentration for degradation. The soluble chemical oxidation demand (sCOD) was used to measure the concentration of 1,4–dioxane at regular time intervals. Furthermore, gas chromatography/mass spectrometry (GC/MS) was used to analyze the presence of intermediate products at the end of the treatment process.

2. Materials and Methods

2.1. Materials

Figure 1 presents an overview of the experimental study into the biodegradation of 1,4–dioxane. The sludge for the study was obtained from a textile wastewater treatment plant located in Gumi, Gyeonbuk, near a river that had been discharging 1,4–dioxane and other organic chemicals (1,4–dioxene, 2–methyl–1,3–dioxolane) for years. The characteristics of the textile wastewater are shown in Table 1. The basal salt medium (BSM) used in this study was prepared according to methodology available in the literature [36,43]. The chemicals used to prepare the BSM were of analytical grade, unless otherwise specified. The 1,4–dioxane (99.9%) was obtained from Sigma-Aldrich.

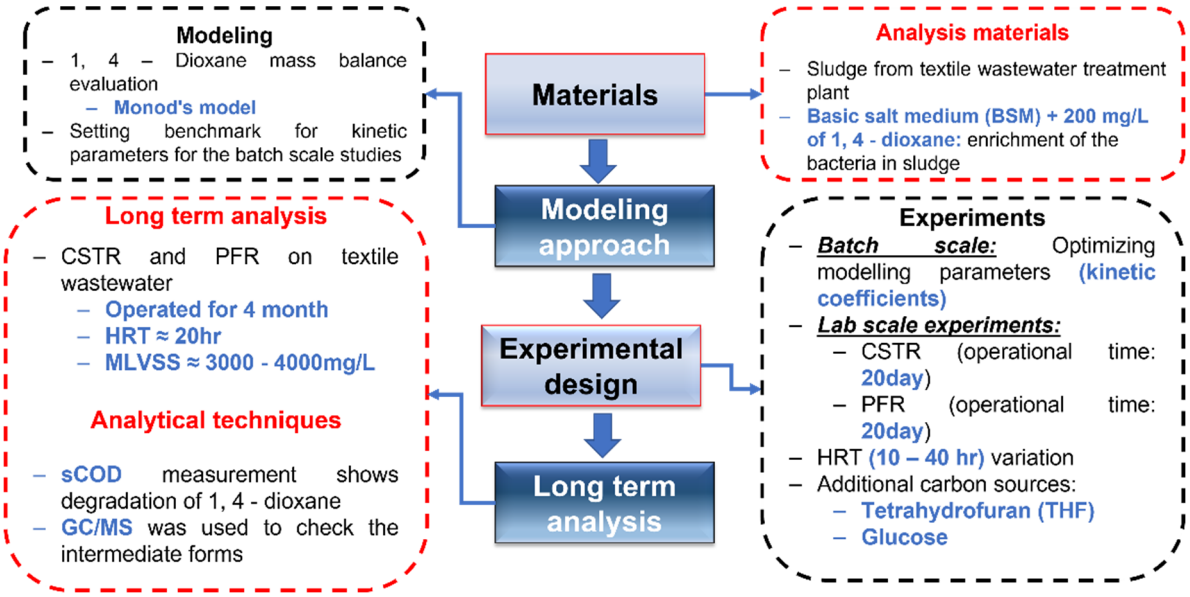

Figure 1. Schematic diagram of the experimental study.

Table 1. Characteristics of industrial wastewater.

Characteristics	Value
1,4-dioxane (mg/L)	185.5–225.5
CODcr (mg O_2/L)	1092–1820
BOD (mg O_2/L)	894–1617
SS (mg/L)	96–155
pH	8.9–9.4
Temperature °C	35.9–37.1

CODcr: chemical oxidation demand (measured by dichromate method). BOD: Biological oxidation demand. SS: Suspended solids.

2.2. Modelling Approach

Biodegradation of 1,4–dioxane has been investigated by various microbial metabolic processes, including those using pure and mixed cultures [46,47]. Efforts to characterize 1,4–dioxane biodegradation have identified both bacterial and fungal strains as useful, including those belonging to the *Rhodococcus strain* [48], *Cordyceps sinensis fungus* [49], *Pseudonocardia* sp. *strain ENV478* [50], *Graphium* sp. [51], *Mycobacterium* sp. *PH*-06 [39] *Afipia* [52,53], *Dokdonella* [53], *Xanthobacter* [26,54] and *Flavobacterium* [3]. However, in this study, a mixed culture was tested with two types of bioreactors, CSTR and PFR, on the lab scale. Moreover, the coefficient of these reactors' kinetic parameters regarding the rate of 1,4–dioxane utilization was determined by applying the Monod model, expressed in Equation (1).

$$\frac{dS}{dt} = -\frac{q_m\, S}{K_s + S}\left[X_a^0 + Y_t\,(S_0 - S)\right] \tag{1}$$

Here, K_s represents the half-saturation coefficients (mg 1,4–dioxane/L), q_m denotes the maximum specific substrate utilization (mg^{-1},4-D/mg-MLVSS/hr), Y_t represents the true cell yield (mg-MLVSS/mg^{-1},4-D), X_a^0 denotes the concentration of the biomass in the initial state (mg/L), S_0 represents the concentration of the substrate in the initial state (mg/L), and S represents the concentration of the substrate at a function of time, t (mg/L). The batch-scale experiments were conducted for the determination of the substrate utilization constants and to estimate the unknown parameters in Equation (1).

The substrate utilization rate depends on the concentration of the active biomass present. One important aspect is that this concentration initially rises, as the active biomass concentration increases asymptotically as it approaches its maximum. Here, K_s represents the half-saturation coefficient (mg, 1,4–dioxane/L) at 0.5 q_{max} due to the decay of the carbon source, as elucidated through the curve-fitting results of the batch study discussed later, similar to earlier work [4].

A batch test was also conducted to determine the decay rate of biomass without the presence of a carbon source and the decay rate of the active biomass, considered to be a first-order reaction. A biotic control was used to determine the loss of the mixed culture, modeled as shown in Equation (2), with the inert biomass fraction ignored [4].

$$\mu_{dec} = \left(\frac{1}{X_a}\frac{dX_a}{dt}\right)_{decay} = -k_d \rightarrow k_d = -\left(\frac{1}{t} \times \ln\left(\frac{X_a}{X_a^0}\right)\right) \tag{2}$$

In this equation, k_d represents the endogenous decay coefficient of the biomass (mg–MLVSS/mg–VSS/day), which can be obtained from the decreasing slope of biomass decay as a function of time. The observed yield strength of cell growth was estimated by linearization of the substrate (1,4-dioxane) utilization rate. This is expressed by Equation (3).

$$Y_{ob} = \frac{dX_a/dt}{dS/dt} = \frac{C_{growth}}{1,4 - Dioxane_{utilization}} \tag{3}$$

As mentioned earlier, a batch test was used in this study to estimate cell yield based on the observation of biomass production measured relative to the substrate used in the test

samples. We also determined the true yield (its relationship with decay rate is expressed by Equation (4) of substrate utilization at different substrate concentrations with a fixed mixed-culture biomass concentration using a methodology found in the literature [4].

$$Y_t = Y_{ob}(1 + k_d SRT) \quad (4)$$

Here, the solid retention time (SRT) is equal to the hydraulic retention time (HRT), which is 48 h. Moreover, the maximum specific microbial growth rate (μ_{max}, day^{-1}) can be determined through the relationship between the coefficients of true yield and substrate utilization rate, expressed as $\mu_{max} = Y_t\ x\ q_{max}$.

Using constant values of yield strength (Y_t) and endogenous decay rate (k_d) in the batch test analysis, variations in K_s and q_{max} can be calculated for a lab-scale analysis of CSTR and PFR for comparison. The substrate degradation rate, Equation (1), is used under various operation conditions of the two bioreactors in order to find the kinetic parameters for scale-up applications over the long term [43].

2.3. Experimental Design

2.3.1. Enrichment of Mixed Culture

The sludge used here was produced from wastewater treatment plants and cultured in BSM (32.4 g K_2HPO_4, 10.0 g NaH_2PO_4. H_2O, 32.4 g K_2HPO_4, 20.0 g NH_4Cl, 2.0 g $MgSO_4$. $7H_2O$, 0.12 g $FeSO_4$. $7H_20$, 0.03 g $MnSO_4$. H_2O, 0.03 g ZnSO4. $7H_2O$, and 0.01 g $CoCl_2$. $6H_20$) at room temperature and incubated with concentration of 200 mg/L of 1,4–dioxane as the sole carbon source (substrate). Moreover, incubation was performed with 1,4–dioxane for 20 days to develop a mixed microbial culture capable of degrading only 1,4–dioxane. The mixed culture completely degraded the 1,4–dioxane to biomass, as in a previous study [4]. Biodegradation was monitored by measuring the sCOD, and once sCOD was depleted, a small portion of the culture was seeded into fresh BSM solution at 50 mg/L of 1,4–dioxane and the process was repeated several times. Moreover, degradation of 1,4–dioxane was also tested for competitive inhibition in the presence of THF, 1,4–dioxene and 2–methyl–1,3–dioxolane using a mixed enzyme culture grown using sludge in BSM. Here, 50 mL of BSM containing various concentrations of 1,4-dioxane was put into a series of 100 mL flasks and inoculated with the 1,4-dioxane culture medium for culturing in a shaking incubator under 25 °C. The initial concentration of the sample was set to 5–1600 mg/L of 1,4-dioxane, and the experiment was conducted until complete degradation at a fixed biomass concentration of 700 mg/L. Moreover, two flasks with enriched culture were analyzed as biotic controls without a carbon source (1,4-dioxane). In addition, to find the optimal conditions for long-term analysis, the degradation of 200 mg/L of 1,4–dioxane in the mixed culture was analyzed under various doses of active biomass in the range of 500–5000 mg MLVSS/L. Experiments were repeated twice, and the samples were incubated at room temperature with a uniform shaking speed of 150 rpm. MLVSS were measured initially and at the end of each experiment according to the standard method to determine cell production outcome. Batch-scale analyses were conducted to determine the kinetic coefficients of biodegradation, and the Monod equations were fitted to the results for the prediction of lab-scale and pilot-scale analyses.

2.3.2. Continuous Stirred Reactor (CSTR)

A continuous stirred tank reactor with a 15 L aeration tank and an 8 L settling tank was designed for the lab-scale tests. The returned sludge was controlled at the optimized biomass concentration for continuous operation with SRT adjustments, as illustrated in Figure 2a. Influent raw water was completely mixed by stirring at 180 rpm, providing aeration to maintain the dissolved oxygen (DO) concentration at 6.1 mg/L. A 1,4-dioxane influent water concentration of 200 mg/L was fixed for degradation at various HRTs in the range of 40–10 h, with an adaptation period of 14 days for each HRT. Moreover, the 1,4–dioxane concentrations in effluents collected in the settling tank were measured through sCOD measurements and GC/MS analysis, and the biomass concentration was measured

through MLVSS measurements for every HRT variation. In addition, fresh 1,4–dioxane solutions were used for a uniform analysis in each case, and stabilized degradation results were reported (initial stages of fluctuations were ignored). The Monod equation model was used to predict the optimized biomass concentration according to the optimized HRT for continuous flow analysis in the CSTR case.

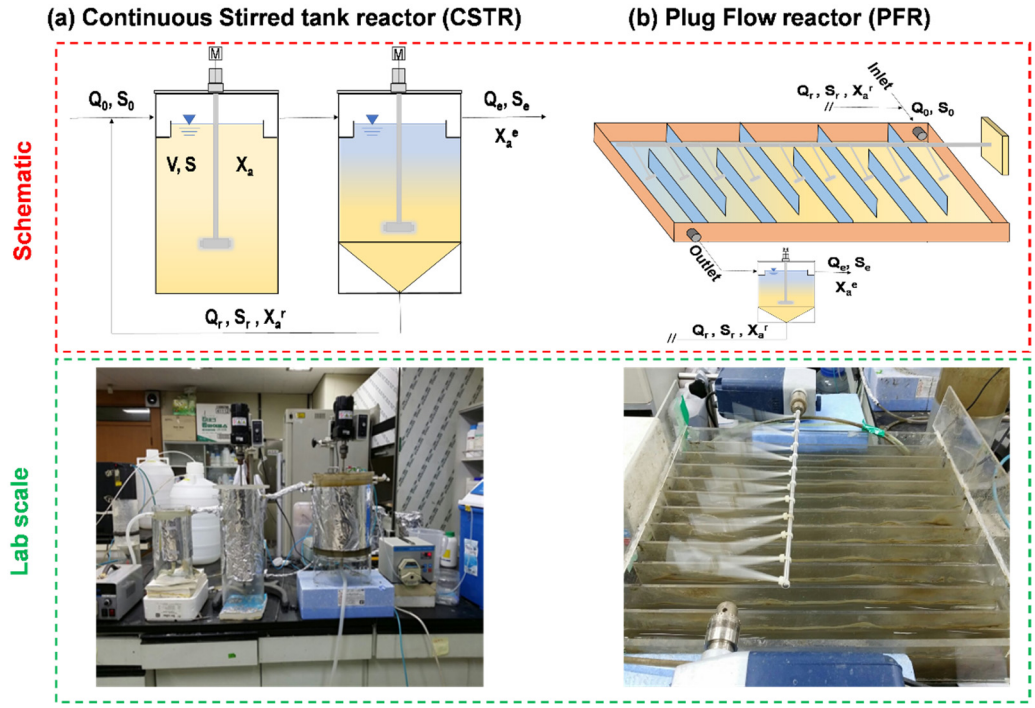

Figure 2. Overview of the lab-scale reactor setups with a schematic for the biodegradation of 1,4–dioxane: (**a**) CSTR and (**b**) PFR.

2.3.3. Plug Flow Reactor (PFR)

A lab-scale PFR reactor with an 11 L capacity and a 6 L settling tank was designed as shown in Figure 2b. The designed reactor was partition-type, with a total flow path of 7 m and with an effective area of 14 m^2. The sludge-recycling and HRT-variation methodology for PFR was closely followed here for comparison of the biodegradation efficacy with a 200 mg/L initial concentration of 1,4–dioxane. In addition, a uniform DO concentration of 6 mg/L was maintained by vertical stirring at a rate of 50 rpm; this also prevents the sludge from settling in the flow path. The slow degradation characteristics of 1,4–dioxane meant that no further aeration was required and that stirring would be enough to provide conditions nearly similar to those of an ideal PFR. The quality of the effluent was analyzed similarly to in the CSTR case, and the adaptation period for the reactor was set to 14 days. However, unlike in the CSTR case, for the PFR, the optimized MLVSS was estimated by testing the reactor at various biomass concentrations ranging from 2000 to 4000 mg/L at the optimized HRT value, with reactor tests also run for continuous analysis. The operation of the lab-scale reactor was tested with real wastewater from the textile industry (the same industry that previously provided the sludge used to culture the mixed culture media) for more than 120 days under optimized conditions, and the results were compared to design an appropriate pilot plant test.

2.4. Analysis Procedure

A gas chromatograph (GS) (Hewlett–Packard Company., Wilmington, NC, USA) equipped with a 5973-mass spectrometer (MS) with a HP-5MS (30 m × 0.25 mm I.D. × 0.25 µm) fused-silica capillary column was used to determine the 1,4–dioxane concentration using the liquid–liquid extraction (LLE) method. This device was also used to test for

the presence of intermediate products and other carbon sources with varying concentrations during the biodegradation of 1,4–dioxane, as mentioned in previous studies [4,43]. The sCOD value was measured using a DR/2010 portable data-logging spectrophotometer (HACH, Loveland, CO, USA). The bacterial concentrations were measured as MLVSS with the standard method [55,56]. In addition, MATLAB (Math Works, Inc., Natick, MA, USA) was used to fit the Monod model to the experimental data and for the prediction of kinetic parameters of the degradation of 1,4–dioxane in each condition for the batch-scale and lab-scale analyses.

3. Results and Discussion

3.1. Batch Test Analysis for 1,4-Dioxane Biodegradation

A batch test reactor was used to determine the endogenous decay of the mixed culture during 1,4-dioxane degradation, and to evaluate the cell yield, as shown in Figure 3. Moreover, Figure 3a illustrates the degradation of the fixed 200 mg/L concentration 1,4-dioxane under various biomass concentrations. The degradation rate increased when the samples were inoculated at a high biomass concentration due to the presence of a very large active microbial community that quickens the process of 1,4-dioxane degradation. The results in Figure 3a indicate that the complete degradation of 1,4-dioxane requires 13.37, 19.96, 39.40 and 76.81 h for MLVSS rates of 3000, 2000, 1000, and 500 mg/L, respectively. Moreover, an experiment was conducted to assess the endogenous rate at VSS = 700 mg/L (by biotic control).

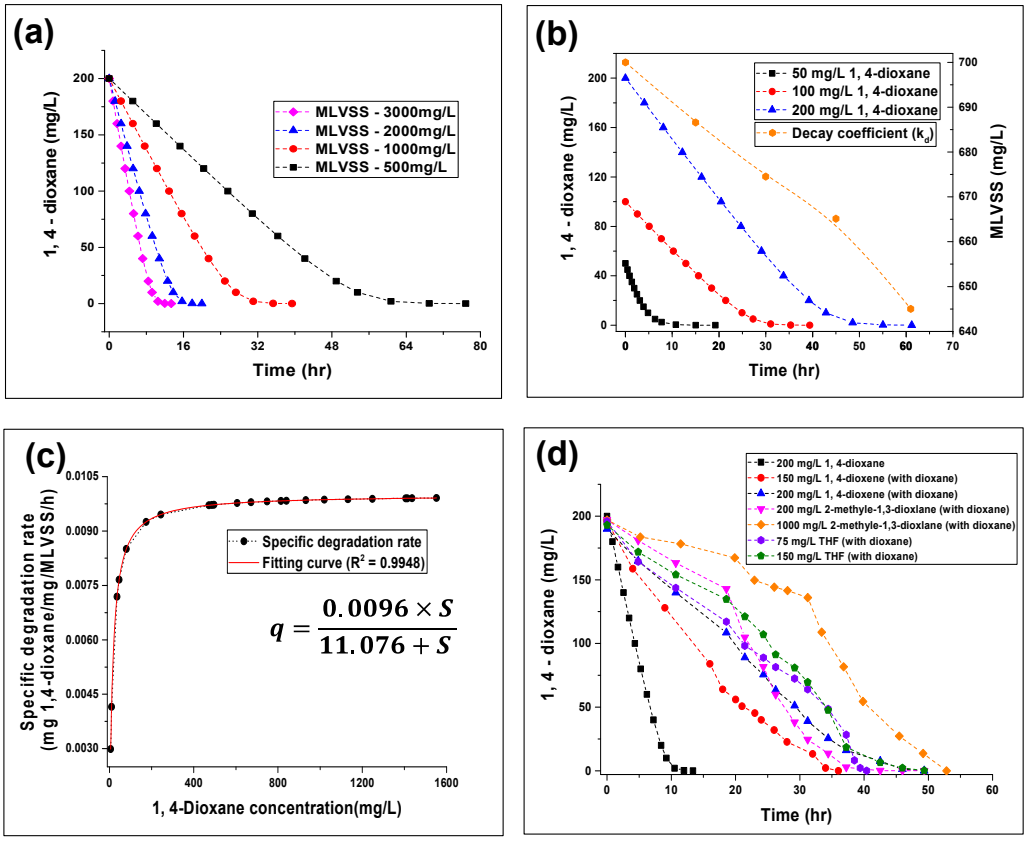

Figure 3. Batch test analysis for 1,4-dioxane degradation: (**a**) impact of the variation of MLVSS on the biodegradation of 200 mg/L of 1,4-dioxane; (**b**) variation in the biodegradation of 1,4-dioxane at a fixed MLVSS level of 700 mg/L for biotic control for determination of the decay rate; (**c**) Monod plots of various kinetic parameters determined at various initial concentrations; and (**d**) impact of a competitive inhibitor on the biodegradation of 1,4-dioxane.

Various doses of 1,4–dioxane were also tested under identical conditions to determine the impact of dose on the time required for complete degradation at a specific dose of the mixed culture grown on BSM sludge media, as shown in Figure 3b. The results showed that an increase in degradation time was observed with an increase in the initial dose of the substrate (1,4-dioxane). Moreover, the specific substrate-degradation rate was used to evaluate the kinetic coefficients of the degradation of 1,4–dioxane, as illustrated in Figure 3c. The results are summarized in Table 2.

Table 2. Summary of kinetic parameter evaluation by batch testing of 1,4-dioxane biodegradation.

Kinetic Parameters	Values
K_s (mg 1,4-Dioxane/L)	11.076
q_{max} (mg 1,4-dioxane/mg MLVSS/h)	0.0096
k_d (mg MLVSS/mg MLVSS/day)	0.03
Y_t (mg MLVSS/mg 1,4-dioxane)	0.432
μ_{max} (day^{-1})	0.099

The specific degradation rate was evaluated for various substrate concentrations ranging from 5 to 1600 mg/L (results not shown) [4]. The Monod equation was fitted to find the K_s and q_{max} values via regression analysis and to find the kinetic coefficients of 11.076 mg substrate/L and 0.0096 mg substrate/mg MLVSS/h. The specific degradation rate found in this study, using the mixed culture grown on BSM sludge, was much lower than in previous studies. A q_{max} value of 1.09 and a rate of 0.1 mg1,4-dioxane/mg protein/h were reported for pure cultures of *Pseudonocardia dioxanivoran CB1190* and *pseudonocardia benzenivorans B5*, respectively [30,31]. Moreover, a q_{max} value of 0.45 mg and a rate of 1,4-dioxane/mg TSS/day were recorded for a mixed culture in the presence of THF as a growth substrate [31]. These results elucidate that the mixed culture grown in the sludge and BSM mixture contains various types of microbial communities that can sustain 1,4-dioxane and serve as a carbon source. Thus, K_s in this study was also relatively low compared to in other studies, supporting the observation that the mixed culture has greater affinity for 1,4-dioxane biodegradation. In addition, the affinity of the mixed culture for the degradation of the 1,4-dioxane was evaluated in a competitive environment (in the presence of the THF, 1,4-dioxene and 2-methyl-1,3-dioxlane), as shown in Figure 3d. A delay in the degradation of 1,4-dioxane was observed in the presence of structural analogs [51,57]. Moreover, the biodegradation of 1,4-dioxane was also inhibited by an increase in the concentration of the competitive inhibitor [26]. The time to remove 1,4-dioxane increased from 13.37 h to 46, 49 and 55 h in the presence of 1,4-dioxene, 2-methyl-1,3-dioxlane, and THF, respectively, whereas the degradation of the competitive inhibitor was not affected (2-methyl-1,3-dioxlane) or increased (1,4-dioxene and THF) under mixed culture inoculation in the presence of 1,4-dioxane. The results in Figure 3d indicate that the competitive inhibition of 1,4-dioxane biodegradation was greater for 2-methyl-1,3-dioxalane than THF and 1,4-dioxene. The enzymes used in the 1,4-dioxane degradation and THF cases are similar, and they have a significant affinity toward THF compared to 1,4-dioxane as a substrate, meaning that the degradation in this case also increased. Similar effects were observed for 1,4-dioxene, where inhibition was more significant in 2-methyl-1,3-dioxlane due to the use of a high concentration [43]. Continuous reactors (CSTR and PFR) were designed and optimized to evaluate 1,4-dioxane biodegradation from raw industrial wastewater containing different structural analogs, which will be discussed later.

3.2. 1,4-Dioxane Biodegradation from Industrial Wastewater

3.2.1. Continuous Stirred Reactor (CSTR) Analysis

The CSTR was operated for a long-term analysis of the biodegradation of 1,4-dioxane based on the design factors determined through the batch test and the model evaluation, as shown in Figure 4. However, the optimum HRT was initially evaluated for 1,4-dioxane biodegradation as the sole carbon source exposed to a mixed culture grown on BSM and

sludge, as discussed in Section 2.3.1, for 20 days of operation at a biomass concentration of 3000 mg/L. The initial concentration of 1,4-dioxane was fixed at 200 mg/L and the HRT was varied from 10 to 40 h, as shown in Figure 4a. The results summarized in Figure 4a indicate that after 20 days of operation in each case, the initial concentration is reduced to 2.8, 2.9, 17.66 and 36.8 mg/L for 40, 20, 15, and 10 h HRTs, respectively. The concentrations of 1,4-dioxane for HRTs of 20 and 40 h were similar at 20 days of operation, showing that an increase in the HRT such that it exceeds 20 h does not influence biodegradation or the enzymes required to biodegrade 1,4-dioxane at the optimum level. Interestingly, the steady-state operation of the CSTR causes the substrate concentrate to reach S_{min} under ideal conditions. The designed kinetic parameter modeling used to evaluate S_{min} shows a concentration of 4.78 mg/L, much higher than the concentration at 20 h, i.e., the optimized HRT. The CSTR was not operated under ideal conditions and the actual concentration of the microorganisms involved in the degradation of 1,4-dioxane was not accurate. The microorganisms of the mixed culture used in this study may explain the difference in the actual and theoretical substrate concentrations.

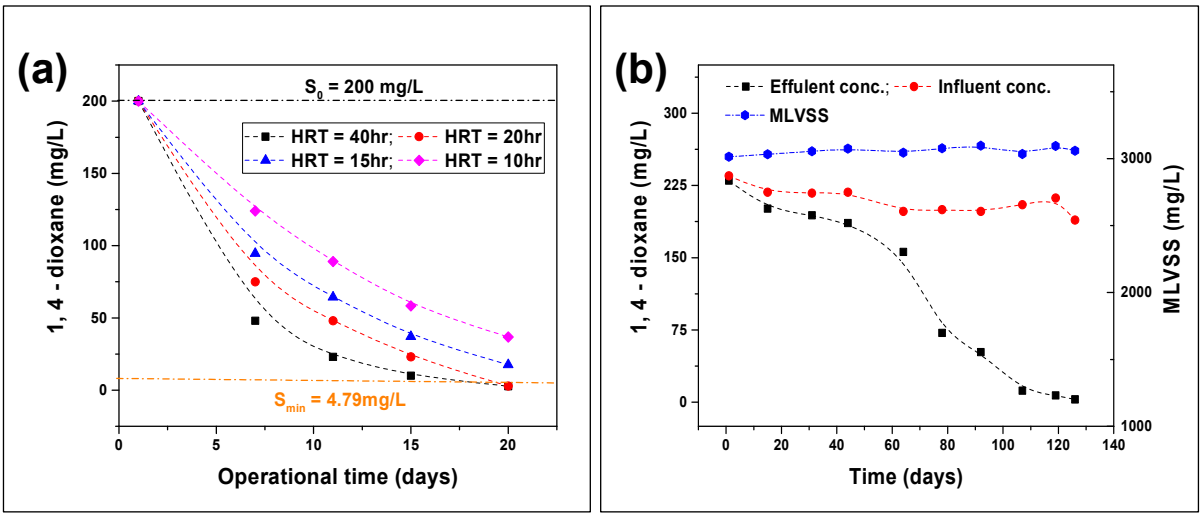

Figure 4. Lab-scale operation of the CSTR plant for 1,4-dioxane biodegradation under a mixed culture grown on the BSM and sludge mixture using 1,4-dioxane as a substrate source: (**a**) HRT optimization at a biomass concentration of 3000 mg/L; (**b**) 1,4-dioxane biodegradation from industrial wastewater under long-term operation with optimized conditions.

Experimental results after using CSTR to process industrial wastewater were also gained for more than 120 days under identical conditions, and biomass and 1,4-dioxane concentrations were evaluated at regular intervals, as shown in Figure 4b. These results show that the biomass concentration in the reactor remains at 3000 mg/L, though a decrease in the 1,4-dioxane concentration was observed, reaching its maximum of 3 mg/L at the end of the study. However, the decrease in the 1,4-dioxane biodegradation rate was due to the presence of the competitive inhibitor, as confirmed in the GC-MS results and as shown in Figure 5a. The competitive inhibitor (2-methyl-1,3-dioxlane) reduces biodegradation as it is readily available and hinders microorganisms that use 1,4-dioxane as a carbon source, as discussed earlier in relation to the batch test. Moreover, the GC/MS results also confirmed that the degradation of 2-methyl-1,3-dioxlane was not affected in the CSTR operational mode. However, the 2-methyl-1,3-dioxlane peak was not displayed in the effluent of the PFR according to the GC/MS results. Moreover, decreases in the peaks of 1,4-dioxane in the CSTR and PFE cases were confirmed, as indicated in Figure 5b,c.

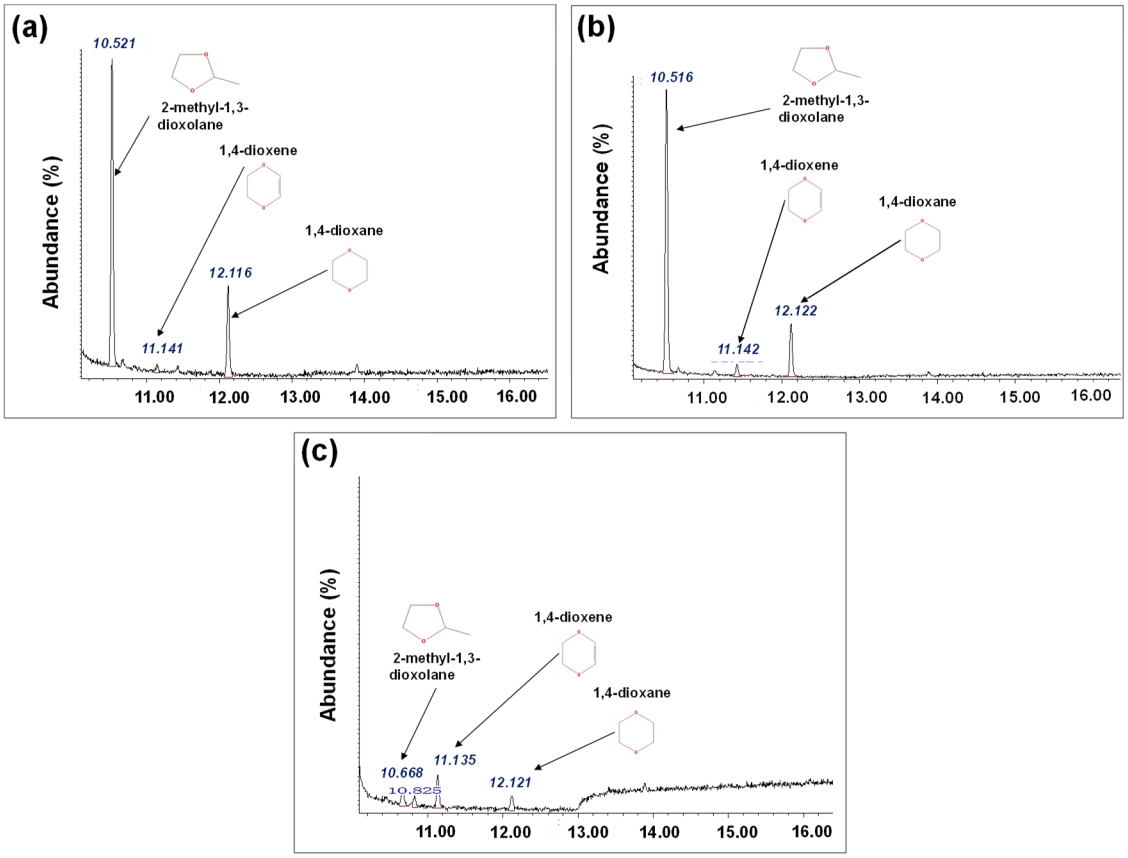

Figure 5. Time-series GC/MS of the wastewater from the textile industry. (**a**) raw wastewater, (**b**) effluent of CSTR, and (**c**) effluent of PFR.

3.2.2. Plug Flow Reactor (PFR) Analysis

The initial conditions for the operation of the PFR were similar to those for the CSTR, and the PFR was operated for 20 days at different HRTs, as shown in Figure 6. The reactor was operated under different conditions to derive the optimum environment in which to operate the PFR for long-term biodegradation of 1,4-dioxane from industrial wastewater. The results in Figure 6a indicate that the concentration of 1,4-dioxane in the effluent was reduced to 0.5, 0.91, 4.68 and 7.69 mg/L at HRTs of 40, 20, 15 and 10 h, respectively. Interestingly, the PFR results also show that the optimum HRT was 20 h when the concentration of 1,4-dioxane was reduced to <1 mg/L. In addition, the biomass concentration was greater in the PFR case when tested for long-term analysis of the industrial wastewater treatment, as illustrated in Figure 6b. A biomass concentration greater than 4000 mg MLVSS/L was recorded in the PFR case, compared to 3000 mg MLVSS/L for the CSTR. Moreover, the industrial effluent results' analysis revealed a 0.4 mg/L concentration of 1,4-dioxane after more than 120 days of operation. Compared to the CSTR, the PFR results in a better 1,4-dioxane biodegradation efficiency in a competitive environment by reducing the degradation level to <1 mg/L. The higher rate of biomass production during the operation of the PFR may be the reason for this higher degradation efficiency. However, controlling the experimental conditions under actual field operations was difficult for the PFR. Accordingly, a dispersive PFR for a pilot plant application is under consideration, and conditions will be optimized for future applications of 1,4-dioxane biodegradation in a mixed culture. The results, in comparison with those in previous studies, are summarized in Table 3. They verify that the PFR, with some modifications to control the experiments, would be a suitable solution for the efficient degradation of 1,4-dioxane in a complex competitive environment.

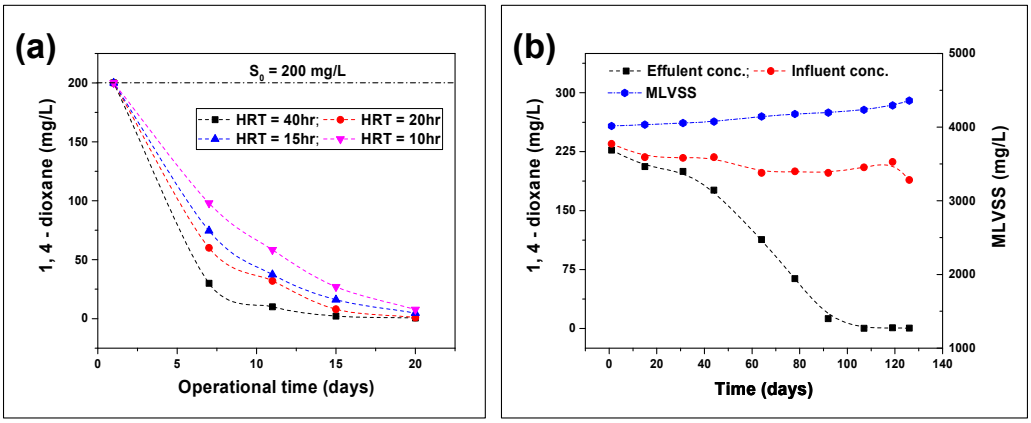

Figure 6. Lab-scale operation of the PFR plant for 1,4-dioxane biodegradation under a mixed culture grown on the BSM and sludge mixture using 1,4-dioxane as a substrate source: (**a**) HRT optimization at a biomass concentration of 3000 mg/L; (**b**) 1,4-dioxane biodegradation from industrial wastewater under long-term operation under optimized conditions.

Table 3. Comparative results summary of the biodegradation of 1,4-dioxane with previous studies based on source wastewater and treatment technology.

Influent Characteristics	Biomass Properties		Kinetic Parameters	Technology	Results	Explanation	Ref.
Source	Conc. (mg/L)	Growth Features	K_S (mg/L), qmax (day^{-1}), k_d (day^{-1})		Conc. (mg/L)		
Synthetic wastewater	100	X. Flavius DT8 (Activated WW sludge)	K_s = 17.5, qmax = 0.42 k_d = 0.073	Batch test	1.83	• The grown enzymes could degrade the other cyclic ethers, including THF. • Several intermediates were formed during degradation including 1,4-dioxene, however, cytochrome 450 s were not responsible for the oxidation of 1,4-dioxane.	[26]
//	50	CB1190	K_s = 160 k_d = 1.1	Batch test	-	• The monooxygenase-expressing strain oxidizing 1,4-dioxane in metabolic and co-metabolism processes. • Dioxane was not degraded by particulate methane, toluene or toluene–2,3–dioxygenase side chain induced by monooxygenase.	[30]
	50	B5	K_s = 330 k_d = 0.1	Batch test	-		
//	100	Cultured grown (Activated sludge)	-	Batch test	0.8	• Enrichment of the media with the THF resulted in degrading the 1,4-dioxane to a non-detectable limit. • However, grown media alone shows in significant degradation efficiency.	[34]
Industrial wastewater	200–300	Ozonation was used for the degradation process and optimized for better operation (pH optimization)			5–65	• O_3 treatment significantly degraded 1,4-dioxane at higher pH (>9) and at higher OCC [1] (>1.5). • MDO [2] significantly acts as the competitive inhibitor and slows down the degradation rate. • O_3 alone is insufficient, while combined treatment (O_3/H_2O_2) efficiently degrades pollutants such as 1,4-dioxane.	[7]
Synthetic wastewater	1.09–1.25	THF substrate (on aquifer)	K_s = 10.8 k_d = 1.09	Trickling filter	0.043–0.078	• A trickling filter can efficiently treat the low-level degradation of 1,4-dioxane. • High THF as a substrate for microbial growth would be more effective for 1,4-dioxane degradation.	[31]
//	6 mM	Actinomycete (pure culture)	-	Batch test	0.55 µM	• The pure culture can effectively degrade THF and MDO along with 1,4-dioxane. • It converted 59% of the carbon from 1,4-dioxane to CO_2 for a long-run operation.	[36]
//	900	Bacterial strain PH-06 (river sediment)	-	Batch test	100	• During degradation of 1,4-dioxane, degradation metabolites (1,4-dioxane-2-ol and ethylene glycol) were identified during turnover experiments. • PH-06 stain is also effective for transformation of THF, 1,3-dioxane and cyclohexane to hydroxylated intermediates along with 1,4-dioxane.	[39]
Industrial wastewater	≈200	Mixed culture (on WW sludge)	K_s = 11.07, qmax = 0.23 k_d = 0.03	CSTR	3	• Mixed microbial culture grown on WW sludge used 1,4-dioxane as a carbon source successfully in the presence of competitive inhibitor. • Biodegradation efficiency of PFR > CSTR at 20 h HRT operation for 120 days.	This study
				PFR	0.4		

[1] OCC: Oxygen equivalent chemical oxidant capacity (1 OCC = 1 g/L of O_3); [2] MDO: 2-methyl-1,3-dioxolane.

The results (CSTR and PFR) indicated that the mixed culture grown on sludge using 1,4-dioxane as a carbon source can be successfully used for the biodegradation of 1,4-dioxane. The lab-scale results from the PFR test analysis show comparatively better performance in the biodegradation of 1,4-dioxane compared to the CSTR and results from other studies, as summarized in Table 3.

4. Conclusions

The goal of this study was to evaluate 1,4–dioxane degradation under a mixed biological culture grown on textile wastewater sludge, and to test its suitability for long-term analysis with a CSTR and PFR for the treatment of textile wastewater. Moreover, the optimized conditions, including the HRT and the MLVSS concentration, were established according to a batch-scale analysis and by using the Monod equation to determine the decay rate and unknown parameters. The following conclusion could be drawn from the results.

- In the batch-test analysis, a relatively low specific substrate utilization rate (q_{max}) of 0.0096 mg of 1,4-dioxane/mg MLVSS/h was observed, with a half-saturation coefficient (K_s) of 11.076 mg 1,4–dioxane/L. Moreover, an endogenous biomass decay rate (k_d) of 0.03 day^{-1} and maximum specific microbial growth rate (μ_{max}) of 0.099 day^{-1} were observed to optimize the degradation of 1,4–dioxane under a mixed culture condition, grown using 1,4–dioxane as the sole carbon source.
- GC/MS results showed that the presence of 2-methyl-1,3-dioxlane as a competitive inhibitor hindered the degradation of 1,4–dioxane. Moreover, the presence of structure analogs, such as THF and 1,4–dioxane increased the degradation time for 1,4–dioxane. However, no changes in the degradation of the inhibitor were observed, but increases in the degradation time were noted, which increased to 55 h from 13 h.
- In a long-term analysis involving CSTR and PFR tests, a HRT of 20 h is considered to as the optimal condition for the efficient degradation of 1,4–dioxane. Moreover, effluent concentrations of 3 mg/L and <1 mg/L of 1,4–dioxane were observed in the CSTR and PFR tests. The higher removal efficacy by the PFR was due to the production of a higher MLVSS level of 4000 mg/L, compared to 3000 mg/L in the CSTR in a competitive environment.

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