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Aerobic biodegradation of lignosulfonate bearing synthetic wastewater using activated sludge

Biswabandhu Chatterjee*, Sanjit Mandal and Debabrata Mazumder

Environmental Engineering Cell, Civil Engineering Department,
Indian Institute of Engineering Science and Technology, Shibpur, Howrah-711 103, West Bengal, India

E-mail: cbiswabandhu@yahoo.com

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Lignin comprises a major part of wastewater emanating from paper and pulping industries. Not only that, appreciable amount of lignin has also been noticed in agricultural waste. Strategies involving treatment of lignin "waste" have been mostly carried out under anaerobic environment. The present study concerns the aerobic biodegradation of lignosulfonate using biomass from municipal wastewater sludge. The acclimation of the biomass was best served using synthetic feed comprising sodium lignosulfonate and dextrose-D, which served as a co-substrate. During acclimation of the biomass, inhibition was noticed in the batch where the synthetic feed had lignosulfonate to dextrose ratio of 1.22. However, the bioreactor system managed to overcome this inhibition and stability was restored during the later batches, which were run at higher lignosulfonate to dextrose ratios. Maximum COD (Chemical Oxygen Demand) removal percentage was recorded as 90.6%, whereas the maximum specific COD reduction rate was observed to be 0.0016 h^{-1}.

Keywords: Aerobic biodegradation, synthetic wastewater, lignin, dextrose, COD removal.

Introduction

Lignin along with cellulose and hemicellulose are not only the principal components of plant material, but they also constitute a major part of the carbon fixed via photosynthesis[1]. Lignin is basically an aromatic polymer made of dehydropolymerized structural units, which form the basis for cohesion between fibers in wood tissues[2]. The dehydropolymerized units are derived from different phenylpropanoid subunits, namely coumaryl, guaiacyl and syringyl moieties[3]. These subunits, as shown in Fig. 1, are essentially monolignols and are also identified as *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol[4].

The ratio of these monolignols varies in different plant species. For example, lignin in softwood comprises coniferyl alcohol, lignin in hardwood comprises both coniferyl and sinapyl alcohols, whereas lignin in grass consists of all the three subunits[5]. The formation of the phenylpropanoid polymers (which are very much resistant to chemical and biochemical depolymerization), in lignin, is mainly on account of the different bonds conjugated by the lignin monomers[4]. Ether linkages, especially β-aryl ether (β-O-4), have been found to be the most dominant in lignin. Hardwood lignin has more of these linkages than softwood lignin.

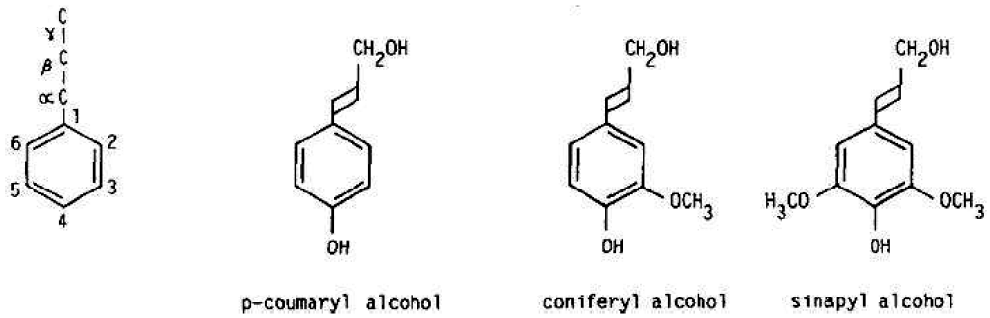

Fig. 1. Carbon nomenclature in lignin, and three monolignols as lignin building blocks (Adopted from Ref. [1]).

Currently, lignin is one of the most abundant naturally occurring polymers, since it not only constitutes a vital part of the biospheric carbon cycle but is also generated in huge quantities from the paper and pulp industries. According to Chen and Wan[4], approximately 50 million tons of lignin "waste", which include Kraft lignin, lignosulfonate, soda lignin, is contributed by the paper and pulp industries annually. Furthermore, courtesy the U.S. Energy Independence and Security Act of 2007, which has mandated the use of 36 billion gallons biofuels by 2022, 21 billion gallons of biofuels are to be produced from lignocellulosic biomass which in turn would generate an estimated 62 million tons of lignin annually. At present, lignin is used as a low-quality solid fuel in bio-refinery industries to produce heat and electricity. However, since lignin is the most abundant renewable feedstock, consisting of aromatics, its valorization, especially by the bio-refinery industries, would not only enable new uses as value-added chemicals and fuels, but would also enable sustenance of a versatile and economical industry that uses lignocellulosic biomass as raw-material[4,6].

Isolation of lignin from lignocellulosic biomass is one of the challenging aspects. One of the categories of isolating lignin from lignocellulosic biomass includes hydrolyzing the cellulose and hemicellulose while keeping lignin as an insoluble residue. Another strategy includes the dissolution of lignin, while leaving cellulose and hemicellulose as insoluble residues, followed by its recovery from the liquor stream[4]. This technique is very much popular in paper and pulping industries where the production of chemical pulp from wood and non-wood materials leads to the generation of lignin, which is degraded and dissolved almost completely (90–95%) in a black liquor[3]. However, removal of lignin from the generated wastewater is a critical aspect since its presence results in toxicity and therefore poses a serious threat to aquatic ecosystems. It is mainly due to its low biodegradability and high range of color that lignin is considered as a serious offender in marine pollution[7]. Many new methods, such as incineration of black liquor lignin[8], the UV/TiO_2 system[9], and chemical coagulation of lignin using synthetic and natural coagulants[10], have been developed for the treatment of lignin emanating from paper and pulp industries. In addition, various adsorbents have been also reported for the removal of color and other pollutants. However, saturation of the adsorbent sites due to high total dissolved solids and total suspended solids in the waste was found to be one of the major limitations in its widespread application[11].

Due to their specificity and cost-effectiveness, biological systems, constituting fungi, actinomycetes and bacteria, have been widely implicated in lignin biodegradation and decolorization of pulping effluent[12]. However, the use of white-rot fungi, which is known for its powerful lignin-degrading enzymatic systems[13], and fungi-based treatment system in general, have been largely found wanting due to the inability of fungi to remain stable under extreme environmental and substrate conditions such as higher pH (which facilitates removal of lignin), alkaline environment, variations in temperature, contamination by spores, high extractive and lignin concentration[14,15]. Biodegradation of lignin via bacteria, on the other hand, has found widespread practical application and deserves to be explored further for ligninolytic potential[16] owing to their superior environmental adaptability and immense biochemical versatility[15]. The present study highlights the performance of an aerobic bioreactor treating synthetic lignosulfonate bearing wastewater using municipal wastewater sludge. Acclimation of the microbes was done via gradual step-up in lignosulfonate concentrations over several batches, and thereafter the lignosulfonate concentration at which inhibition occurred was also found out.

Experimental

Materials and methods:

The acclimation study was carried out using a lab-scale setup (aerobic bioreactor), which consisted of a 7 L cylindrical plastic container and two aerating units (shown in Fig. 2).

The aerating units were two single-motor fitted aquarium pumps, which were placed inside the aforesaid plastic container. Biomass for the acclimation study was collected from a two-year old lab-scale suspended aerobic bioreactor (of 10 L volume), treating municipal wastewater. After homogenizing the entire content, 5 L of biomass from the old aerobic bioreactor was procured and the acclimation study was initiated. The acclimation study was undertaken at the Environmental Engineering Laboratory in IIESTS (Indian Institute of Engineering Science and Technology, Shibpur), and the bioreactor was kept in room temperature (24 to 30°C).

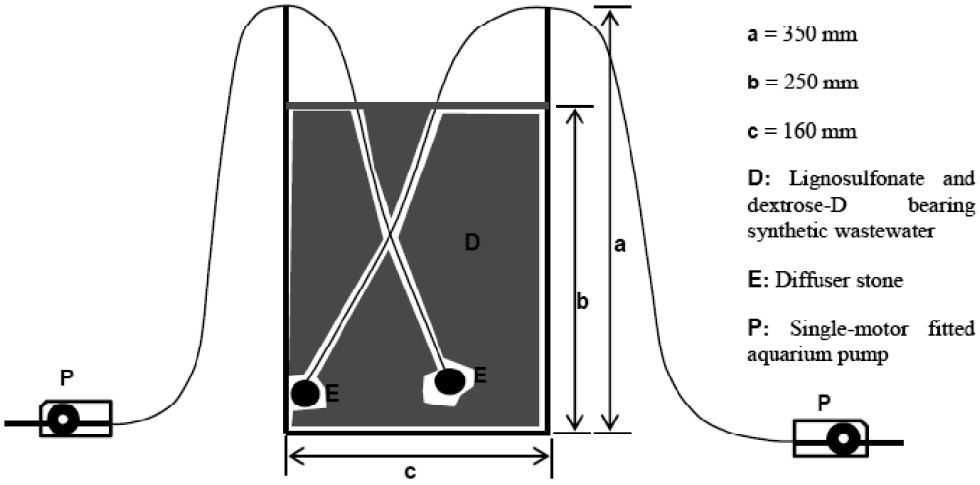

Fig. 2. Schematic diagram of the aerobic bioreactor treating lignosulfonate bearing synthetic wastewater.

Since dextrose-D was used as the co-substrate during the course of the entire acclimation phase, two types of synthetic feed, dextrose-D bearing synthetic feed and sodium lignosulfonate bearing synthetic feed, were prepared as specified in Table 1 and Table 2. The C:N:P ratio in both the feed solutions were kept at 100:5:1.

The biomass was gradually acclimatized to degrading lignosulfonate bearing synthetic feed. This was achieved under batch mode of operation using 100 mL of synthetic feed comprising both dextrose-D and sodium lignosulfonate bearing synthetic solutions, respectively. During the onset of each batch, suitable volume of supernatant from the bioreactor was replaced by a predetermined volume (100 mL initially, and later on up to 160 mL) of the fresh synthetic feed. At the beginning of the acclimation study, batches were initiated using a combination of 95 mL of dextrose-D bearing synthetic feed and 5 mL of lignosulfonate bearing synthetic feed. In the subsequent batches thereafter, a 5 mL step-up in the volume of the lignosulfonate bearing synthetic feed was done in till the microbes achieved degradation of 100% lignosulfonate bearing synthetic feed i.e. 100 mL of the synthetic feed entirely comprised lignosulfonate. In the initial stages of the acclimation study, each combination of sodium lignosulfonate to dextrose-D bearing synthetic feed was run only once. This was done till the sodium lignosulfonate to dextrose bearing synthetic feed had reached a ratio of 25:75 (i.e. 100 mL synthetic feed comprising 25 mL sodium lignosulfonate bearing synthetic feed and 75 mL dextrose-D bearing synthetic feed). Mid-way through the batch operations, some of the batch combinations had to be run multiple times since there was pronounced inhibition in the bioreactor performance. The durations of the batches varied between 4 days (96 h) to 6 days (144 h) depending on the biodegradation of the introduced synthetic feed. The batches were continued even after the acclimation phase i.e. beyond feed volume of 100 mL, in order to ascertain the maximum specific

Table 1. Composition of dextrose bearing synthetic feed

Sl. No.	Reagents used	Amount (g/L)
1.	Dextrose-D anhydrous ($C_6H_{12}O_6$)	10.0
2.	Ammonium chloride (NH_4Cl)	0.77
3.	Potassium di-hydrogen phosphate (KH_2PO_4)	0.15
4.	Ferric chloride ($FeCl_3$)	0.25
5.	Magnesium sulfate ($MgSO_4$)	22.5
6.	Calcium chloride ($CaCl_2$)	27.5
7.	Phosphate buffer ($KH_2PO_4.K_2HPO_4$)	0.15

Table 2. Composition of sodium lignosulfonate bearing synthetic feed

Sl. No.	Reagents used	Amount (g/L)
1.	Sodium lignosulfonate ($C_{20}H_{24}Na_2O_{10}S_2$)	8.9
2.	Ammonium chloride (NH_4Cl)	0.77
3.	Potassium di-hydrogen phosphate (KH_2PO_4)	0.15
4.	Ferric chloride ($FeCl_3$)	0.25
5.	Magnesium sulfate ($MgSO_4$)	22.5
6.	Calcium chloride ($CaCl_2$)	27.5
7.	Phosphate buffer ($KH_2PO_4.K_2HPO_4$)	0.15

COD (Chemical Oxygen Demand) reduction rate. This was achieved with 10 mL increase in the (lignosulfonate bearing) synthetic feed volume thereafter.

For monitoring the bioreactor performance Chemical Oxygen Demand (COD), Mixed Liquor Suspended Solids (MLSS) and pH were measured at 24 h interval as per APHA Standard Methods[17]. For this purpose, 50 mL of sample was collected after homogenizing the entire content of the bioreactor. After collecting the sample, its pH was recorded using an Orion 420A+ (Thermo Electron Corporation) pH meter. Thereafter the sample was passed through a pre-weighed commercial filter paper following which the filtrate was measured for soluble COD via closed reflux dichromate method[17]. The filter paper was then oven dried at 105ºC, following which it was brought to room temperature and transferred into a desiccator. Weight of the filter paper, containing the oven dried biomass, was noted thereafter. In order to ensure precision, duplicate samples were taken every time and analyzed for all of the above three parameters.

Results and discussion

In addition to the 20 different combinations of lignosulfonate to dextrose-D bearing synthetic feed (starting from: 95 mL dextrose-D bearing synthetic solution plus 5 mL lignosulfonate bearing synthetic solution to 5 mL dextrose-D bearing synthetic solution plus 95 mL lignosulfonate bearing synthetic solution with 5 mL step-up in lignosulfonate bearing synthetic feed, and finally 100 mL of lignosulfonate bearing synthetic solution), multiple trials of some of the intermediate batches had to be conducted during the course of the entire acclimation phase. This was done since in the initial stages there was pronounced inhibition, as observed from Figs. 3, 4 and 6.

In order to overcome the inhibition, multiple trials corresponding to a single batch had to be performed, alongside addition of suitable pH buffers (2 *N* $NaHCO_3$ and 1 *N* HCl) and different micro-nutrients. Results from 15 typical batches, which include the following lignosulfonate (L) to dextrose-D (D) combination: L (30 mL) + D (70 mL), L (45 mL) + D (55 mL), L (55 mL) + D (45 mL), L (60 mL) + D (40 mL), L (65 mL) + D (35 mL), L (75 mL) + D (25 mL), L (80 mL) + D (20 mL), L (85 mL) + D (15 mL), L (90 mL) + D (10 mL), L (95 mL) + D (05 mL), L (100 mL) + D (00 mL), L (110 mL), L (120 mL), L (140 mL) and L (160 mL), have been shown.

It can be observed from Fig. 3 that during the initial stages of acclimation (as shown in case L (30) + D (70) and L (45) + D (55) batches) the pH remained around 7.0 to 7.4. This is attributed to the easily biodegradable nature of the co-substrate dextrose, whose decomposition was not only achieved easily but led to degradation of the major fraction (since the

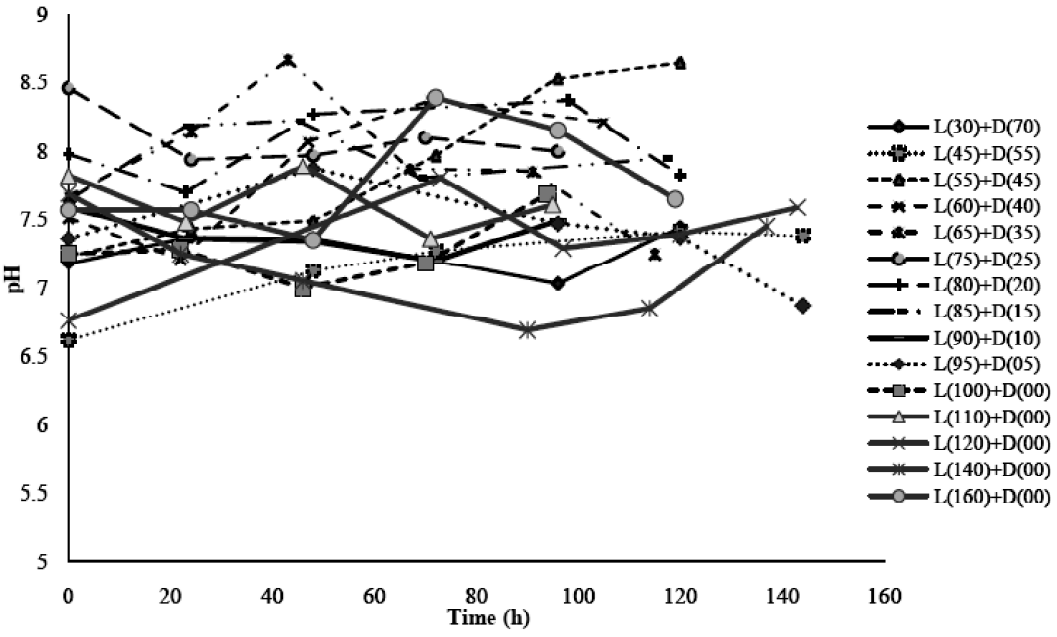

Fig. 3. pH profile for various lignosulfonate to dextrose ratios.

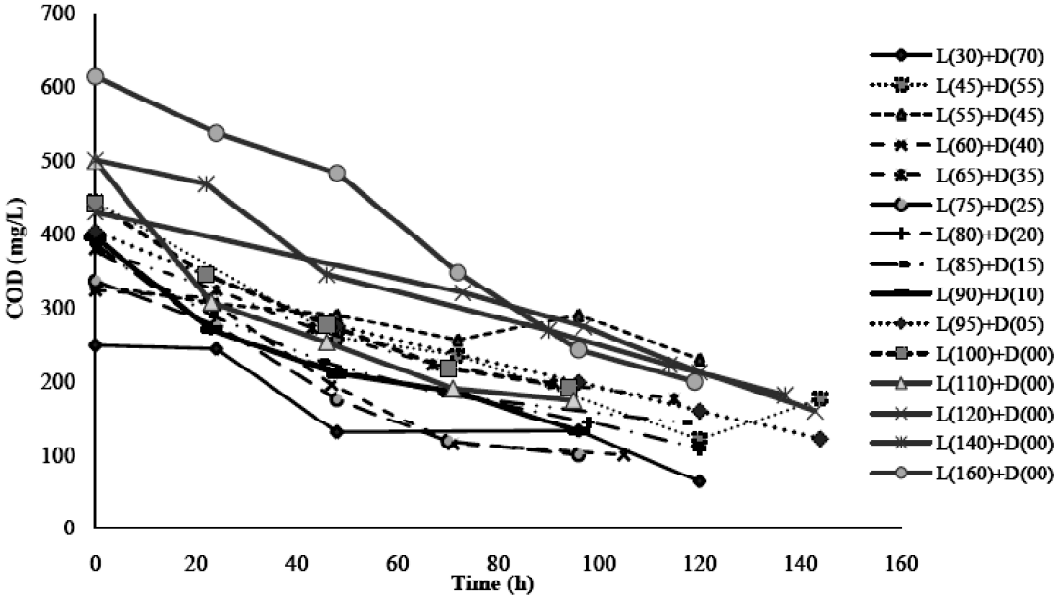

Fig. 4. COD profile for various lignosulfonate to dextrose ratios.

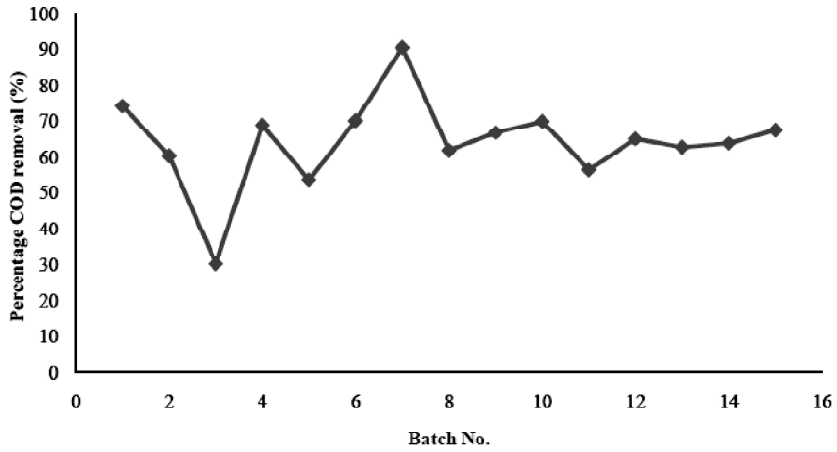

Fig. 5. Profile of percentage COD removal in various batch runs.

fraction of dextrose had been higher in the initial batches) of the feed provided (in case of a single batch). Inhibition was noticed, with the pH getting significantly above 8 at the end of almost all the batches (from batch L (55) + D (45) to batch L (85) + D (15)) once the lignosulfonate to dextrose ratio exceeded 1. As such, multiple trials for numerous batch combinations had to be conducted, and at the start of every batch HCl buffer solution had to be added in order to restore the system pH within the desirable range (7–7.5). It can be observed that once the acclimation phase had reached beyond batch combination L (90) + D (10), the pH got stabilized. The increase in pH beyond 8 can be attributed to the ligninolytic activity of the microbes that were responsible for the degradation of lignosulfonate.

Usually degradation of lignin, under both aerobic and anaerobic environment, involves lowering of system pH[18]. Under aerobic environment, bacterial degradation of lignin proceeds via breakdown of β-ether linkages to phenolic hydroxyl groups, and formation of α-carbonyl groups as a consequent to oxidation of propyl side chains, demethylation and aromatic ring cleavage[1]. In addition, microbial degradation of lignin compounds proceeds with the release of substan-

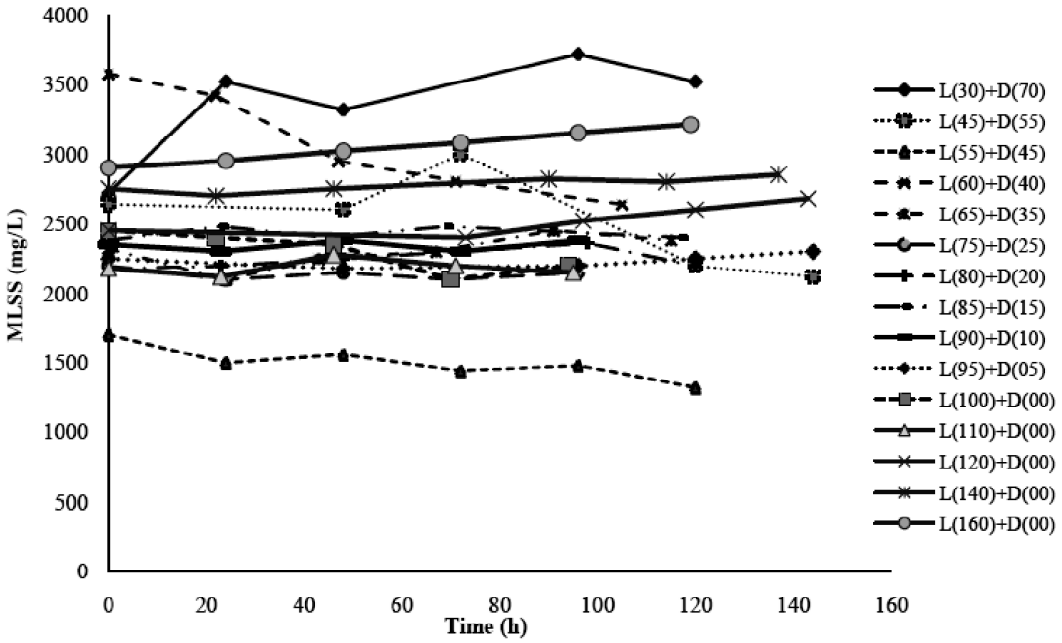

Fig. 6. MLSS profile for various lignosulfonate to dextrose ratios.

tial amount of CO_2 and certain water soluble products. Normally, generation of CO_2, α-carbonyl groups, and phenolic hydroxyl groups lower the system pH[1]. Furthermore, it has been observed that most of the common and popular microbial species (white-rot fungi, *Acinetobacter* sp., *P. chrysoporium*) known to degrade lignin exhibit optimum/desirable activity under slightly acidic (pH 4.0–5.0) condition[1,18]. However, the enzymatic activities of microorganisms are largely dependent on the condition under which the microorganisms have been adapted to. The mixed microbial cultures that are present in municipal wastewater sludge, and have been used in the present study exhibited optimum enzymatic activity in the pH range 7–7.5. Also, it has been revealed by various past studies that the pH-requirements for polymer transformation/degradation are very likely to differ with different lignin degrading microbial-groups[1,19–21].

As such in the present study, the increase in pH beyond 8 may have been on account of optimum ligninolytic activity of the microbes, degrading lignin, beyond pH 7.5. Furthermore, it was observed by Chandra *et al.*[22] that during aerobic biodegradation of black liquor wastewater (containing high amounts of lignin), collected from pulp and paper manufacturing industry, the depletion of supplementary nutritional source, such as calcium, magnesium, nitrogen and phosphorus, resulted in rising of the medium pH beyond 8. The rise in pH had in essence facilitated the biodegradation of lignin, since according to several past studies[22–24] high pH favors solubility of lignin and its subsequent derivatives. The lignin-degrading microbes growing in the black liquor, as observed in the present study and in the study reported by Chandra *et al.*[22], necessitated high amounts of dissolved oxygen in alkaline condition for improved degree of COD degradation (as shown in Fig. 4).

It can be observed from Fig. 5 that during the initial stages when the lignosulfonate to dextrose-D ratio was gradually increased above 0.42 there was a pronounced drop in the COD removal rates, with the percentage COD reduction dropping to as little as 30.3% in case of the combination L (55) + D (45). However, the recovery occurred soon after from the next combination L (60) + D (40) onwards, with the highest COD reduction recorded as 90.6% in case of the combination L (80) + D (20). The COD reduction percentage got stabilized at the end of the acclimation phase, and for batches thereafter. Following the acclimation phase, the highest specific COD removal rate was recorded in case of batch 12, L (110) + D (00), as observed from Table 3.

Thereafter, the specific COD removal rate trend became asymptotic, and the batch runs were terminated due to the inability of the microbes to reduce the final COD, at the end of 144 h, below 200 mg/L.

Table 3. Results of the acclimation runs in the aerobic bioreactor

Batch type	Initial COD (S_0)	Final COD (S)	Batch period (T)	COD reduction per hour	Initial MLSS (X_0)	Final MLSS (X)	Average MLSS (X′)	Specific COD reduction rate
L(30) + D(70)	249	64	120	1.54	2720	3520	3360	0.0005
L(45) + D(55)	444	176	144	1.86	2640	2120	2512	0.0007
L(55) + D(45)	330	230	120	0.83	1700	1320	1500	0.0006
L(60) + D(40)	323	100	105	2.12	3575	2640	3078	0.0007
L(65) + D(35)	380	176	115	1.77	2320	2380	2300	0.0008
L(75) + D(25)	336	100	96	2.46	2200	2150	2140	0.0012
L(80) + D(20)	387	108	120	2.33	2180	2200	2238	0.001
L(85) + D(15)	378	144	117	2.00	2380	2400	2428	0.0008
L(90) + D(10)	396	131	96	2.76	2350	2380	2342	0.0012
L(95) + D(05)	403	121	144	1.96	2250	2300	2236	0.0009
L(100) + D(00)	441	192	94	2.65	2450	2200	2300	0.0011
L(110) + D(00)	499	174	95	3.42	2180	2150	2186	0.0016
L(120) + D(00)	430	160	143	1.89	2450	2680	2530	0.0008
L(140) + D(00)	501	181	137	2.34	2750	2850	2779	0.0008
L(160) + D(00)	614	199	119	3.49	2900	3210	3052	0.0011

COD in mg/L; MLSS in mg/L; Batch period in h; Specific COD reduction rate = $[(S_0 - S)/ (X'T)]\ h^{-1}$.

With the increase in percentage of lignosulfonate in the synthetic feed, the microbes had to adapt to consuming primarily the lignosulfonate very soon from the onset of a particular batch (with lignosulfonate to dextrose-D ratio greater than 0.42) since the glucose in the synthetic feed got depleted very fast, unlike in the initial batches where the percentage of glucose in the synthetic feed was noticeably greater.

Unlike in the initial batches (where the lignosulfonate to dextrose-D ratio were less than 0.42), the microbes had to decompose lignin in an environment devoid of any easily biodegradable substrate (in case of batches with lignosulfonate to dextrose-D ratio greater than 0.42). This resulted in the breakdown/conversion of the polymers/macro-molecules into soluble monomers, whose concentrations got reflected in the measured COD values[25,26] thereby dropping the COD removal percentage. As the microbes adapted to the change in the predominant-substrate type present in the synthetic feed, the degradation of the accumulated monomers occurred rapidly in the subsequent batches, leading to an abrupt increase in the COD removal percentage soon after (noticed in case of batch 7, as shown in Fig. 5).

It can be also observed from Fig. 4 that in case of batches 2 and 3 (with lignosulfonate to dextrose-D combinations L (45) + D (55) and L (55) + D (45)) the degradation of COD in the initial 24 h was rapid followed by a noticeable decrease in the COD reduction rate in the remainder of the batch period. The rapid decrease in COD levels during the initial hours of the aforementioned batches was due to the consumption/degradation of the easily biodegradable organics, which were present together with lignosulfonate. However, as observed from Fig. 6, and contrary to observations noticed in past studies[21], the rapid decrease in the COD levels was not accompanied by a sharp increase in MLSS levels. The MLSS concentrations remained stable, except for batch 3, mostly throughout the entire acclimation phase and thereafter. After the conclusion of batch 3, which exhibited inhibition of the bioreactor, microbes had to be added in the range of approximately 2000 mg/L from the aforesaid 10 L aerobic bioreactor in order to counter the inhibition due to accumulation of monomers. Apart from addition of microbes from outside, the pH also had to be lowered by the addition of 1 *N* HCl. Furthermore, micro-nutrient supplementation (1 mL/$L_{reactor}$) was also done. All of the above countered the inhibition and successfully stabilized the bioreactor.

The present study corroborated the fact that addition of microbes from the outside, along with the supplementation of micro- and macro-nutrients, led to improvement of lignin

bio-degradation[1]. The ligninolytic activity of microbes has been reported to get triggered under carbohydrate-limiting environment[1]. The plausible effects include increase in extracellular nitrogen concentration, which in turn represses and interrupts degradation of lignin, and decreased microbial metabolism. As a consequence, the effective microbial populations are sure to experience setbacks, as indicated by the lowering of MLSS concentrations in the present study, due to decreased substrate intake (reduced COD degradation). Several past studies have also revealed the occurrence of catabolite repression[23,27,28] in case of studies/experiments where an easily biodegradable substrate (co-substrate) was used along with lignin. The sudden decrease in MLSS concentration (noticed in case of batches 2 and 3 from Fig. 6) may have been due to the reluctance of the microbes, which predominantly grew up consuming glucose, to take-up/degrade the lignin present in the synthetic feed, once the ratio of lignosulfonate to dextrose-D in the synthetic feed crossed 0.42.

Conclusions

It can be concluded from the present study that the microbial consortia present in municipal wastewater sludge are capable of degrading lignin under aerobic condition. Acclimatization of the biomass can be achieved by slowly increasing the load of lignosulfonate in the synthetic feed, which also comprised dextrose as co-substrate, over several batches. At the beginning of the acclimation phase when the lignosulfonate to dextrose ratios in the synthetic feed were in between 0.05 and 0.43, no inhibition was noticed. At lignosulfonate to dextrose ratio of 1.22 a significant degree of inhibition was noticed, as observed from the COD removal and the MLSS profiles. This inhibition was however, overcome and the batches thereafter showed improved percentage COD removal and consistent MLSS concentrations even at high lignosulfonate to dextrose ratios[19].

References

1. J. A. Buswell, E. Odier and T. K. Kirk, *Critical Reviews in Biotechnology* , 1987, **6(1)**, 1.
2. S. A. Lisboa, D. V. Evtuguin, C. P. Neto and B. J. Goodfellow, *Carbohydrate Polymers*, 2005, **60(1)**, 77.
3. R. Chandra, A. Raj, H. J. Purohit and A. Kapley, *Chemosphere*, 2007, **67(4)**, 839.
4. Z. Chen and C. Wan, *Renewable and Sustainable Energy Reviews*, 2017, **73**, 610.
5. A. Duval and M. Lawoko, *Reactive and Functional Polymers*, 2014, **85**, 78.
6. T. Vangeel, W. Schutyser, T. Renders and B. F. Sels, *Topics in Current Chemistry*, 2018, **376(4)**, 30.
7. D. Berryman, F. Houde, C. DeBlois and M. O'Shea, *Chemosphere*, 2004, **56(3)**, 247.
8. P. Harila and V. A. Kivilinna, *Water Science and Technology*, 1999, **40(11-12)**, 195.
9. C. N. Chang, Y. S. Ma, G. C. Fang, A. C. Chao, M. C. Tsai and H. F. Sung, *Chemosphere*, 2004, **56(10)**, 1011.
10. H. Ganjidoust, K. Tatsumi, T. Yamagishi and R. N. Gholian, *Water Science and Technology*, 1997, **35(2-3)**, 291.
11. V. K. Gupta, I. Ali, V. K. Saini, T. Van Gerven, B. Van der Bruggen and C.Vandecasteele, *Industrial and Engineering Chemistry Research*, 2005, **44(10)**, 3655.
12. F. Perestelo, M. A. Falcon, A. Carnicero, A. Rodriguez and G. De la Fuente, *Biotechnology Letters*, 1994, **16(3)**, 299.
13. A. Hatakka, *FEMS Microbiology Reviews*, 1994, **13(2-3)**, 125.
14. Y. Wang, Q. Liu, L. Yan, Y. Gao, Y. Wang and W. Wang, *Bioresource Technology*, 2013b, **139**, 113.
15. N. Kamimura, S. Sakamoto, N. Mitsuda, E. Masai and S. Kajita, *Current Opinion in Biotechnology*, 2019, **56**, 179.
16. R. Xu, K. Zhang, P. Liu, H. Han, S. Zhao, A. Kakade, A. Khan, D. Du and X. Li, *Bioresource Technology* , 2018.
17. A. APHA, WPCF, Standard methods for the examination of Water and Wastewater, American Public Health Association, Washington, DC, 1995.
18. B. Chatterjee and D. Mazumder, *J. Indian Chem. Soc.*, 2018, **95(3)**, 295.
19. T. Higuchi, M. Shimada, F. Nakatsubo and M. Tanahashi, *Wood Science and Technology*, 1977, **11(2)**, 153.
20. M. J. Barder and D. L. Crawford, *Canadian Journal of Microbiology*, 1981, ***27*(8)**, 859.
21. S. Ban, M. Glanser-Šoljan and M. Smailagi, *Biotechnology and Bioengineering*, 1979, **21(11)**, 1917.
22. R. Chandra, A. Abhishek and M. Sankhwar, *Bioresource Technology*, 2011, **102(11)**, 6429.
23. R. Vicuña, *Enzyme and Microbial Technology*, 1988, **10(11)**, 646.
24. A. L. Pometto and D. L. Crawford, *Appl. Environ. Microbiol.*, 1986, **52(2)**, 246.
25. F. Kariminejad, Z. Yousefi and J. Y. Cherati, *International Journal of Environmental Science and Technology*, 2018, **15(4)**, 719.
26. W. Wang, H. Han, M. Yuan, H. Li, F. Fang and K. Wang, *Bioresource Technology*, 2011, **102(9)**, 5454.
27. D. L. Kaplan and R. Hartenstein, *Soil Biology and Biochemistry*, 1980, **12(1)**, 65.
28. T. J. Kerr, R. D. Kerr and R. Benner, *Appl. Environ. Microbiol.*, 1983, **46(5)**, 1201.