Biodegradation study of cellulose bearing synthetic wastewater in activated sludge system

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Due to the inert nature of cellulose, decomposition of cellulose present in food processing industry wastewater and digestate generating from organic solid waste treatment units is a key aspect in abetting global industrial pollution. The present study describes the performance of an aerobic bioreactor treating cellulose bearing synthetic wastewater. Complete acclimation with cellulose bearing synthetic wastewater was achieved within 10–12 weeks of initiating the acclimation batch runs. Maximum removal of the introduced chemical oxygen demand (COD) was recorded to be 70.4% mid-way through the acclimation phase. An average COD removal percentage in between 64% and 67% was noticed at the end of the acclimation phase. Inhibition of the bioreactor was noticed when the percentage of cellulose in the synthetic wastewater was increased above 70%. This resulted in sharp fall in system pH along with pronounced lowering of MLSS and percentage COD removal. Stability in the reactor performance was however, restored with the adjustment of pH and supplementation of micro-nutrients at suitable levels.

Keywords: Carboxymethyl cellulose (CMC), aerobic biodegradation, synthetic wastewater, COD removal, acclimation study.

Introduction

One of the major components of plant biomass, cellulose can be defined as a carbohydrate made of linear polymeric glucose sub-units that are linked via β-1,4 bonds[1]. Each one of the glucose sub-units or residues is rotated 180° relative to their neighbors. As a result, cellobiose remains as the basic repeating subunit[2]. Fig. 1 shows the typical structure of a cellulose chain. Cellulose chain, whose length varies between 100 and 14000 residues, combines to form several intra- and inter-molecular hydrogen bonds, which results in the formation of rigid and insoluble type microfibrils[1]. The lateral dimensions of microfibrils range from 3–4 nm in higher plants to 20 nm in *Valonia macrophysa*, an algal species comprising several hundred cellulose chains. These chains are characterized by crystalline domains, which remain in highly ordered and parallel form, interspersed by disorderly, amorphous regions[1,3]. Unlike starch, whose role is to serve as a storage polymer for glucose in plants, cellulose, owing to its high tensile strength, serves as the structural unit in plants. Withstanding high osmotic pressure and providing resistance against any sort of mechanical stress are among the key structural attributes of cellulose in plants. This is es-

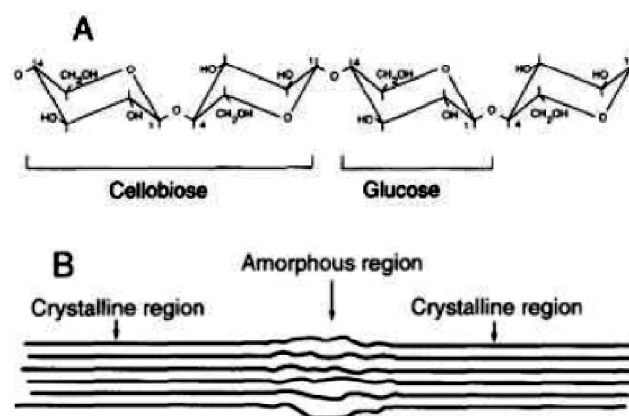pecially evident in case of wood and textile fibers, which comprise elongated and empty cell walls[1].

Fig. 1. Structure of cellulose (A) β-glucosidic bonds (B) schematic structure of a fibril (Adopted from [2]).

As much as 50% of the dry weight of plant biomass is made of cellulose[4]. Since production of plant biomass, via photosynthesis, is continuous and unending, degradation of cellulose represents a crucial part of the carbon cycle within the biosphere[1,3]. About 50% of the dry weights of other sec-

ondary biomass sources, such as agricultural, industrial and domestic wastes, also comprise cellulose[5]. In addition, cellulose is also produced by some bacteria and tunicates[3]. Cellulose, due to its abundance[3,6] can therefore be considered as one of the major sustainable sources of bio-fuel[1–5]. Commercially available pure cellulose forms, which are primarily used for assessing the efficiency of complete cellulase systems, include cotton, filter paper and Avicel[1]. However, treatment of the cellulosic biomass recalcitrance is one of the major challenges researchers have faced over the years. The diversity and heterogeneity in cellulosic structures can be hold responsible for the difficulties surrounding enzymological degradation of cellulose. Furthermore, a rudimentary understanding of the hydrolysis process during the breakdown of different cellulosic substrates has impeded any further improvement in the treatment strategies involving cellulose[3].

In nature, the microbial utilization of cellulose is observed to occur mostly in the guts of termites and in the rumen of ruminant creatures, whose major source of dietary protein comes from cellulose[3]. Cellulolytic enzymes secreted by certain microbes have been observed to be the reason for the aforesaid degradation of cellulose in nature. As such, studies have confirmed that the most robust strategy to overcome the difficulties associated with the breakdown of cellulose should constitute a system of cellulolytic microbes or microbial consortium, where production of cellulolytic enzymes, hydrolysis of cellulosic biomass, and fermentation of the produced sugars to desired products should synergistically occur together[7]. In addition, it has also been reported that degradation of cellulosic material is also possible via mixed culture of cellulolytic bacteria and non-cellulolytic bacteria[1]. All these strategies to ultimately degrade cellulose have drawn the interests of biotechnologists; as such continuous developments in the field of cellulose biodegradation to value-added end products are constantly underway.

In the recent past, various researchers have explored the possibility of producing bio-hydrogen from cellulosic waste by using co-cultures of cellulolytic and dark fermentative hydrogen producing microbes[8,9]. Since cellulose is one of the most abundantly available and cheap resources, bioenergy production from cellulosic waste would always be economically viable. Certain photofermentative bacteria, such as purple non-sulfur photosynthetic bacteria (e.g. *Rhodobacter*

and *Rhodopseudomonas*), have been found capable to utilize organic acids, sugars, and varieties of agricultural and industrial wastes, producing bio-hydrogen as endproduct[10–12]. However, one of the major hurdles towards such production of bio-hydrogen is the reasonable conversion of cellulose to significant yields of soluble substrates, and further conversion to hydrogen[13]. Certain pretreatment techniques, such as enzymatic pretreatment and acid hydrolysis, have been found to be useful in this regard, however, there are quite a few disadvantages that accompany the aforesaid pretreatment techniques, such as production of inhibitory intermediate compounds (furfural) during acid hydrolysis, and use of high enzyme loads (which ultimately escalates the overall cost of treatment) with an aim to increase cellulose conversion[7]. In this regard, it has been asserted by numerous studies that co-culturing of microbes (cellulolytic and photosynthetic bacteria) that break down cellulose firstly, and produce hydrogen from the generated residue secondly, is the best way for *in situ* cellulose degradation and synergistic bio-hydrogen production. The reduced compounds and organic acids, which get produced as a result of incomplete oxidation of sugars during dark fermentation and serve as a dark fermentative effluent (DFE), can be further treated before discharge[7].

Biodegradation of cellulose in nature can occur either aerobically or anaerobically. Anaerobic biodegradation accounts for only 5–10% of the cellulose degradation in nature, and anaerobic protozoa and slime molds have been found to be responsible for this[18]. Together with eubacteria, fungi, other cellulolytic microorganisms and non-cellulolytic species, these establish synergistic relationship to completely degrade cellulosic waste and release CO_2 and H_2O under aerobic condition, and CO_2, CH_4 and H_2O under anaerobic condition[19]. The microbes capable of degrading cellulose produce a wide variety of enzymes, which despite bearing different specificities, work synergistically to breakdown cellulosic waste. The products generated, as a result of cellulosic biodegradation, serve as essential carbon and energy sources for the cellulolytic microorganisms and other different microbes present in cellulosic waste environment[18,19]. Aerobic microbes use the free cellulase mechanism to degrade cellulose whereas brown rot fungi rely on oxidative mechanism to digest cellulose[14]. In the free cellulase mechanism, microbes' secret individual cellulases that contain carbohydrate-binding molecule (CBM) connected to the cata-

lytic domain by a flexible linker[15]. Enzymes present in this mixture act synergistically, with their specific activity being more than 15 times the specific activity of any single cellulase system.

The fundamental difference between aerobic and anaerobic biodegradation of cellulose lies in the type of enzyme systems being carried by the two different species[16]. Aerobic bacteria and fungi consist of non-complex cellulase systems from where enzymes are released into culture medium for cellulose hydrolysis, whereas anaerobic bacteria and fungi comprise complexed cellulase systems where membrane-bound enzyme complexes (cellulosomes) contain the enzymes. The complexed cellulase systems in case of anaerobic microbes find better biotechnological applications compared to their aerobic counterparts, since the consumption of energy is very low compared to the aerobic microbes and thus can be utilized in low-cost bioremediation projects[16]. For instance, hydrolysis of organic solid waste[22] comprising significant lignocellulosic fraction remains uncompleted without the application of proper enzymes. Aerobic sludge, which is recovered from municipal wastewater treatment plant, is believed to comprise microbial communities that are capable of degrading variety of wastes including complex carbohydrates, such as cellulose and lignin. As such, mixing of aerobic sludge that is capable of decomposing cellulosic fraction present in the organic solid waste would eventually result in amelioration of the overall anaerobic digestion process[17]. The present paper focuses on the performance of an aerobic bioreactor, comprising aerobic sludge obtained from a municipal wastewater treatment hybrid lab-scale unit, treating cellulose bearing synthetic wastewater.

Experimental

Materials and methods:

The acclimation study was undertaken at the Environmental Engineering Laboratory of IIESTS (Indian Institute of Engineering Science and Technology, Shibpur) by procuring biomass from a lab-scale aerobic bioreactor, of volume 10 L and being run at the aforementioned laboratory, treating municipal wastewater. After complete mixing, 5 L content from the aforesaid aerobic bioreactor was collected in a 7 L plastic container, which served as the cellulose degrading bioreactor (Fig. 2). Two single-motor fitted aqua-pumps were used for aerating the collected biomass content. For feeding purpose, dextrose-D (anhydrous) and carboxymethyl cellu-
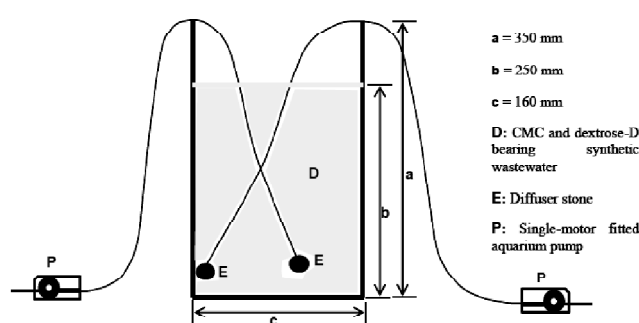

a = 350 mm

b = 250 mm

c = 160 mm

D: CMC and dextrose-D bearing synthetic wastewater

E: Diffuser stone

P: Single-motor fitted aquarium pump

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

lose (CMC) 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.

Table 1. Composition of dextrose bearing synthetic feed

Sl. No.	Reagents	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 carboxymethyl cellulose bearing synthetic feed

Sl. No.	Reagents used	Amount (g/L)
1.	CMC ($C_8H_{16}O_8$)	10
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

Under batch mode of operation, the collected aerobic biomass fraction was gradually acclimatized to degrading CMC. 100 mL composite feed, which consisted of both dextrose-D and CMC bearing synthetic solution, was introduced into the bioreactor at the start of each batch after removing 100 mL supernatant. The feed composition in each batch was adjusted in such a way that there was a gradual increase (by 5

mL) in the ratio of CMC bearing synthetic feed to dextrose-D bearing synthetic feed. At the start of the batch operation, each combination of CMC to dextrose-D bearing synthetic feed was run only once. This was done till the CMC to dextrose bearing synthetic feed had reached a ratio of 25:75 (i.e. 100 mL synthetic feed consisting of 25 mL CMC bearing synthetic solution and 75 mL dextrose bearing synthetic solution). Mid-way through the batch operations, multiple trials for each combination had to be run since there was pronounced inhibition in the bioreactor performance. The durations of the conducted batches varied between 96 h and 144 h based on the biodegradation percentage of the introduced synthetic feed.

Reactor activity was ascertained by monitoring Chemical Oxygen Demand (COD), Mixed Liquor Suspended Solids (MLSS) and pH. These parameters were measured, as per APHA Standard Methods[20], at 24 h interval in order to assess the bioreactor performance. Sample collection, for the purpose of measuring the aforesaid parameters, was done after homogenizing the content of the bioreactor. 50 mL of sample was collected and passed through a pre-weighed commercial filter paper following which the filtrate was measured for soluble COD via closed reflux dichromate method[20]. Prior to filtration the pH was noted using an Orion 420A+ (Thermo Electron Corporation) pH meter. The filter paper was oven dried at 105°C following which it was brought to room temperature and thereafter kept in 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

Results from 8 typical acclimation batch runs, which included the following cellulose (C) to dextrose-D (D) combination: C (55 mL) + D (45 mL), C (70 mL) + D (30 mL), C (75 mL) + D (25 mL), C (80 mL) + D (20 mL), C (85 mL) + D (15 mL), C (90 mL) + D (10 mL), C (95 mL) + D (05 mL) and C (100 mL) + D (00 mL), for synthetic wastewater feed comprising cellulose to dextrose ratios in between 1.22 and 19, and 100 mL of cellulose bearing synthetic wastewater have been presented. It can be observed from Fig. 3, where COD profiles of the aforesaid batches are shown, that inhibition occurred during batches 2 and 3, when the CMC concentration in the synthetic feed had been increased to 70% and

above. This is also asserted by Fig. 5 and Fig. 6, where it can be noticed that there had been an abrupt decrease in percentage COD removal along with a sharp fall in MLSS concentrations.

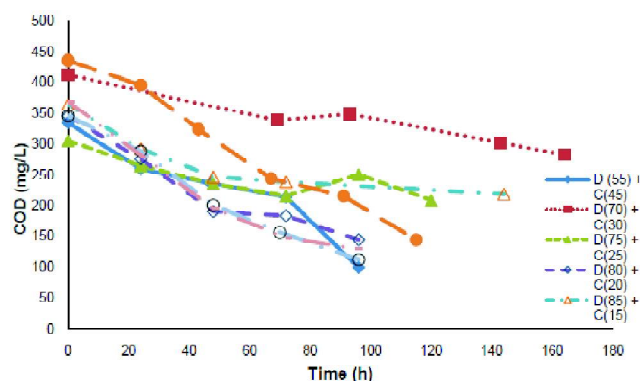The performance of the bioreactor, however, got stable
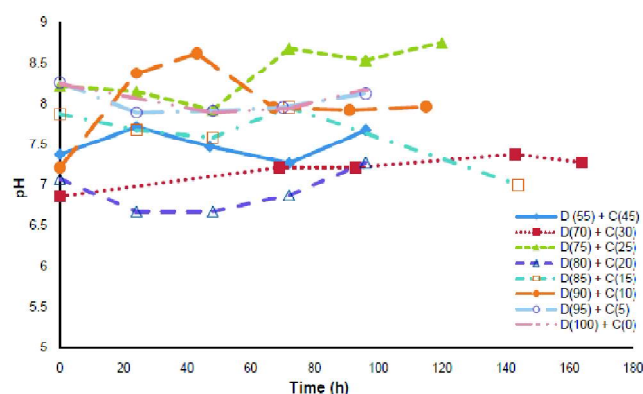

Fig. 3. COD profile for various CMC to dextrose ratios.

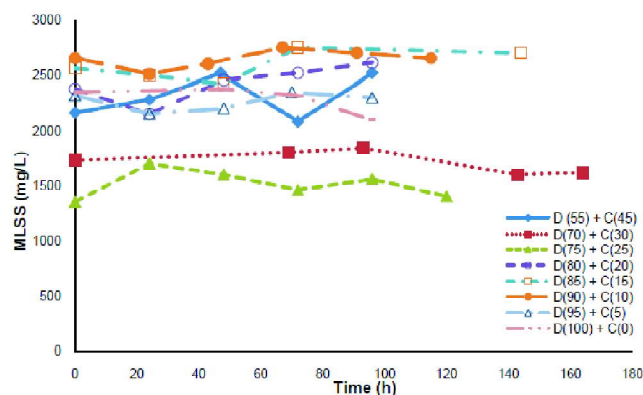**Fig. 4.** pH profile for various CMC to dextrose ratios.

Fig. 5. MLSS profile for various CMC to dextrose ratios.

with the onset of batch 4, which had cellulose to dextrose ratio of 4. The gradual fall in system pH, as observed from Fig. 4, had to be adjusted every time with 2 N NaHCO$_3$ solution. In addition, supplementation of micronutrients (cobalt, nickel, molybdenum, copper, manganese) in suitable amounts had to be done several times in order to counter any possible inhibition. Once the system got stabilized, the pH for the batches from 5 to 8 remained within 7.9 to 8.6, and complete acclimation with 100 mL cellulose bearing wastewater was achieved. This is evident from percentage COD removal profile, shown in Fig. 6, which shows degradation of the introduced COD reaching an asymptotic pattern from the sixth batch onwards, where cellulose to dextrose ratio was 9. The percentage COD removal for the last set of batch runs (including those which had not been shown) with cellulose to dextrose ratio ≥ 9 consistently remained above 64%. Not only that, in case of the batch where only 100 mL cellulose bearing synthetic wastewater feed was introduced, 64.7% COD removal was achieved, as noticed in Fig. 6.
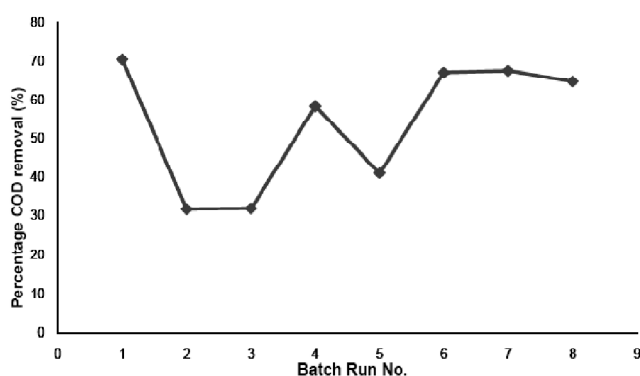

Fig. 6. Profile of percentage COD removal in various batch runs.

Aerobic biodegradation of a polysaccharide, such as CMC, usually proceeds with hydrolysis of the polysaccharide to monosaccharide. This results in the formation of a variety of intermediates including glucose and glycolic acid[21]. The acclimatization becomes faster and easier, with the formation of easily biodegradable intermediates, when carried out with a co-substrate, such as glucose. It is evident from the above shown COD, pH and MLSS profiles, that there was no production of any major bio-stable intermediate, which could have inhibited the acclimation phase. As pointed out in past studies[14,19,21], the most likely products of aerobic CMC degradation are alcohols, ketones, acids (including salts and

esters) and aldehydes, which are readily biodegradable. Past studies have revealed that CMC degradation by aerobic microbes involves hydrolytic scission of the cellulose chain followed by oxidation of the subsequent products[21]. As seen from Fig. 5, the consistent MLSS readings from batch 4 (cellulose to dextrose ratio 4) onwards, only corroborates the evidence from past studies that even if there were production of certain bio-stable intermediates, such as ethers and glycols, the biomass were able to degrade those. This was more even noticeable when micro-nutrient supplementation was done.

Conclusions

It can be concluded from the present study that aerobic biomass obtained from municipal wastewater treatment plant is capable of degrading cellulose bearing synthetic wastewater after proper acclimation. The acclimation phase can be enhanced by using glucose as a co-substrate. Inhibition started to occur when the percentage of cellulose in the synthetic feed was increased above 70%. Countering this inhibition, which was observed from the fall in system pH, was managed by supplementation of various micro-nutrients and adjusting the system pH with suitable buffer solution (NaHCO$_3$ in the present study). Thus for a successful acclimatization process involving the biodegradation of a polysaccharide, certain physical and chemical requirements/standards pertaining to pH, biomass concentration, nutrient supplementation, temperature, oxygen and moisture level ought to be met. In addition, it can also be concluded that the acclimatization is fast achieved, within 10–12 weeks, when coupled with a highly biodegradable co-substrate, such as glucose.

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