Microorganism Degradation Efficiency in BOD Analysis Formulating a Specific Microbial Consortium in a Pulp and Paper Mill Effluent

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Pulp and paper mills are a major source of pollution, generating huge amounts of intensely colored effluent that goes to the receiving end of a wastewater treatment plant. The biochemical oxygen demand test (BOD_5) relies heavily on the microorganism metabolic capability added to the test as seeding material. The seeding material in the testing is obtained from sewage sampling or from commercial sources. Specific organic pollutants that are present in paper and pulp mill effluent can only be degraded by specific microbes; therefore, common sewage or synthetic seed may lead to erroneous BOD_5 estimations. In this study, specific microbial species were selected to evaluate their degradation efficiency, both individually and in combination. The microorganisms selected in the formulated seed exhibit BOD_5 in a reproducible and synergistic manner. The formulation of this specific microbial consortium can be used to develop bioremediation strategies.

Keywords: Paper and pulp; BOD_5; COD; Degradation; Formulation

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INTRODUCTION

The pulp and paper industry contributes heavily to water pollution, which generally arises from the industrial use of fiber, cellulose, and lignin; these components can impart high COD (chemical oxygen demand), color, and BOD to the effluent (Singh *et al*. 2008). The BOD_5 testing is not specific to any pollutant, but can be used as an aggregated water quality parameter indicating the amount of biodegradable organic materials in terms of oxygen consumption (Pepper *et al*. 1996).

Most of the industrial effluents have the necessary microorganisms to realize the BOD_5 test without adding a microbial seed to increase its efficiency. The oxygen used by the microorganism to degrade the organic matter is measured by the BOD_5 and changes with time and concentration. Using microorganisms obtained directly from the process *via* isolation and acclimation improves the treatment (Kumar *et al*. 2010).

Prabu and Udayasoorian (2005) reported that white rot fungus that was isolated from soil samples, enriched by continuous pulp and paper mill effluent irrigation, and identified as *P. chrysosporium*, was capable of 79% COD reduction. De Olivera *et al.* (2009) evaluated the ability of *Bacillus pumilus* CBMAI0008 to produce alkaline enzymes when isolated from wood decomposition and *Paenibacillus* sp. CBMAI 868 production ability when isolated from pulp and paper mill wastewater to produce alkaline enzymes. These bacteria have the uncanny ability to remove color and COD from paper mill effluent.

In mechanistic studies, the oxygen demand is based on the conversion of organic carbon (carbonaceous phase) to CO_2 in the course of 5 days at 20 ° C in complete darkness, by the following reaction,

$$\underline{\text{Organic matter}} \rightarrow NH^{+4} + H_2O + CO_2 + \underline{\text{bacterial biomass}}$$

$$\rightarrow CO_2 + \underline{\text{Protozoan biomass}} \qquad (1)$$

for which the results depend not only on the reaction but on the microorganisms involved (Kumar *et al*. 2010).

Pulp and paper effluents contain lignocellulosic biomass, which is composed of cellulose, hemicellulose, and lignin. Cellulose is the most abundant biopolymer in nature. Three phases of cellulose degradation are distinguished (Charpentier 1968), as indicated below:

$$\text{Cellulose (crystalline)} \rightarrow \text{cellulose (amorphous)}$$

$$\rightarrow \text{cellobiose} \rightarrow \text{glucose} \qquad (2)$$

There are many microbial consortiums capable of cellulose hydrolysis (Hawkes, *et al*. 2002). Some microorganisms such as bacteria (*Bacillus* spp., *Cellumomonas* spp., *Clostridium* spp., and *Streptomyces* spp.,), certain filamentous fungi (*Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp., and *Fusarium solani*), and Protozoa (Marquina 2005) are good sources of hydrolytic activity.

The research aimed to determine the predominant fungi and bacteria present in pulp and paper mill effluent as well as to evaluate the degradation efficiency of each individual microbe selected. In addition, this study performed an analysis of the microbial consortium (native microorganisms capable of degrading cellulose) that was specifically formulated for use as seeding material for the of pulp and paper industrial wastewater BOD_5 and compared it with a commercial formulation prepared by Bio-PAPPEL (Alken Clear-Floc®700S). The initial strains were used to name the formulation (Saraswathi and Saseetharan 2010).

EXPERIMENTAL

Materials

Microorganisms

The strains used in this study originated from the Laboratory of Biotechnology culture collection at the Durango Institute of Technology. These strains were isolated from a paper company at an aerated lagoon. Microorganisms used in this study were the bacteria *Arthrobacter agilis* GenBank accession number KJ790149, *Bacillus licheniformis* KJ790150, *Bacillus seohaeanensis* KJ790151, and *Cellulomonas cellasea* KJ790152 and the fungi *Aspergillus niger* and *Penicillium* sp. The effluent, from the same aerated lagoon, was transported to the laboratory and stored at 4 °C until it was used.

Methods

The physicochemical characterization stage of the testing, which included COD (dichromate) and BOD_5 analysis, was performed according to the Standard Methods for Examination of Water and Wastewater, APHA (Clesceri *et al*. 1998). The BODTrak^{TM} method (Hach 2013) was used to evaluate the treatment efficiency of individual microbes both individually selected and in combination. All the microorganisms selected were screened individually for their ability to serve as a seeding material for BOD_5 analysis.

Efficiency of individual microorganisms and consortium

The bacteria selected and the inoculum Polyseed were used as a seeding material in the BOD_5 analyses. They were individually inoculated in 50 mL of nutrient-rich broth containing 0.01% Polysorbate 80. All of the cultures were incubated at 37 °C for 18 h in an incubator shaker at 120 rpm. The optical density of all of the cultures was maintained at 620 nm, and serial dilution cycles were performed to determine the viable count for the $1x10^8$ cells per mL for each experiment. The bacterial cells were harvested by centrifugation at 5000 rpm for 18 min at 4 °C. The pellet was washed twice with 50 mL of a phosphate buffer solution, pH 7. The process continued by immersing the cells in 7.5 mL of the same buffer, vortexing for 38 s, and was concluded by centrifugation at 5000 rpm for 20 min at 4 °C. The pellet of the individual bacteria was resuspended in 5 mL of the same buffer, and used as seeding material for the BOD_5 analysis of the pulp and paper industrial effluent. After verifying the BOD removal efficiency of each microorganism, the best individual fungi and bacteria were selected to integrate into a formulation.

The microorganisms found to exhibit values comparable to the Bio-PAPPEL sewage-synthetic seeding material were selected for the microbial consortium formulation. The formulated microbial consortium that gave the desired BOD_5 results was tested. Effluent from pulp and paper was previously sterilized to ensure that only the selected microorganisms were added during formulation and of the inoculum Polyseed. The effluent was treated using the selected bacteria and fungi. The treatment efficiency was validated by calculating the removal percentage of the physiochemical parameters. To evaluate the degradation efficiency of the selected individual, formulation experiments of the selected were performed, as shown in Table 1.

Table 1. Experimental Design of Formulations

Formulation	*KJ790150 B. licheni-formis*	*KJ790152 C. cellasea*	*KJ790149 A. agilis*	*KJ790151 B. seohaea-nensis*	*ITDOD021 A. niger*	*ITDOD022 Penicillium* sp.
Control	------	-------	-------	--------	------	--------
LCN	*X*	*X*	--------	--------	*X*	---------
LCP	*X*	*X*	-------	--------	------	*X*
LAN	*X*	-----	*X*	--------	*X*	---------
LAP	*X*	-----	*X*	-------	------	*X*
LSN	*X*	-----	-----	*X*	*X*	---------
LSP	*X*	-----	-----	*X*	-----	*X*
CAN	-------	*X*	*X*	-------	*X*	--------
CAP	------	*X*	*X*	-------	------	*X*
CSN	------	*X*	------	*X*	*X*	-------
CSP	------	*X*	------	*X*	-----	*X*
Note: CFB (Commercial Formulation of Biopappel, Alken Clear-Floc®700S) $1x10^8$ cells/mL of each strain was added to the formulation						

RESULTS AND DISCUSSION

Microbial Treatment of Effluent

The paper and pulp effluent physiochemical data are given in the Table 2. The effluent was treated using bacteria and fungi.

Table 2. Physiochemical Analysis of the Paper and Pulp Effluent (aerated lagoon) (Clesceri *et al*. 1998)

Parameter	Initial value	Range
Temperature (°C)	25	40*
pH	7.3	5-10*
Turbidity (NTU)	423	10**
Color (Pt-Co)	940	75**
Chemical oxygen demand (COD) (ppm)	1134	------
Biochemical oxygen demand (BOD_5) (ppm)	329	75*
Total Suspended Solids (TSS) (ppm)	670	75*
***SEMARNAT (2003) ****CONAGUA (2013)		

In the physicochemical analysis section of Table 2, it can be seen that the parameters of both the turbidity and color show limits that were above those listed in CONAGUA (2013), with 10 NTU and 75 Pt-Co, respectively. Both the BOD_5 and TSS tests performed well above the adequate range defined by SEMARNAT (2003) and CONAGUA (2013), with results of 75 ppm in both. In order to determine the overall biodegradation, the oxygen consumption was measured continuously throughout the experimental period. This process takes place for 5 consecutive days, according to the BODTrak™ method (Hach 2013).

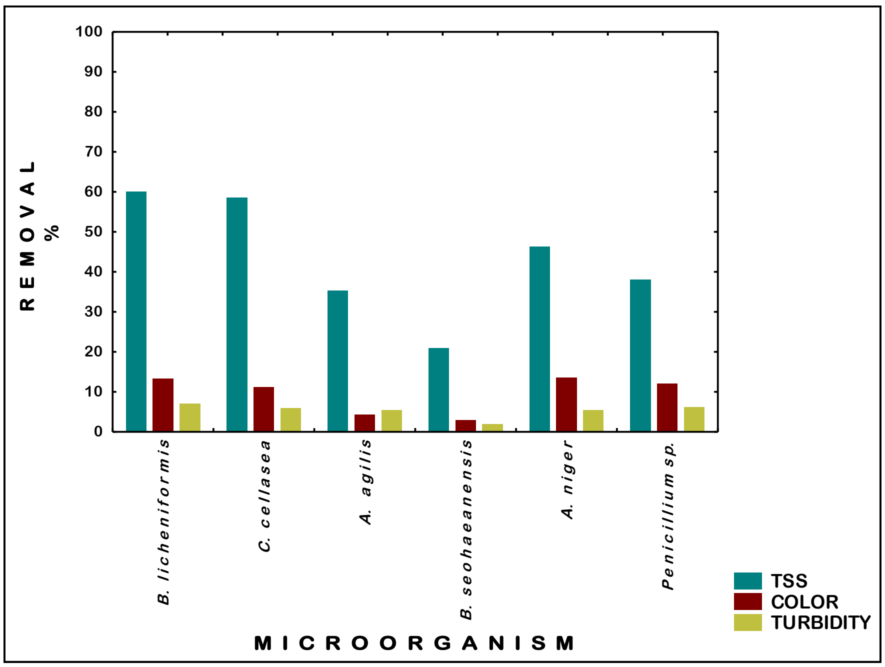

Fig. 1. Removal of TSS, turbidity, and color using the selected bacteria and fungi

In Fig. 1 the reduction of parameters such as turbidity, color, and testing for the selected strain (TSS) are shown. It should be noted that the TSS was not reduced to the adequate level 50 ppm in accordance with the CONAGUA (2013). The bacterium *B. licheniformis* successfully was reduced to 268 ppm at its upper limit. Regarding color, *B. licheniformis* was able to reduce it to 815 Pt-Co (14%), compared to a limit of 75 Pt-Co (CONAGUA 2013). Also, the turbidity was 393 ppm (8%) for the same strain, with a limit of 10 NTU. Nagarathnama and Bajpai (1999) successfully treated the kraft mill effluent using *Rhizopus oryzae* and removed 92 to 95% of the color. According to Kataoka (2001), the biodegradation of organic compounds is most effective when the microorganisms in the inoculum are pre-selected and become potentially more adapted to degrade pollutants.

In Fig. 2, the analysis showed a decrease in BOD_5 quantities after biodegradation reduction in all tests compared with the initial value of Table 2. Good results were obtained with the bacterial strains *B. licheniformis* BOD_5 (75%) and COD (78%), and *C. cellasea* BOD_5 (70%) and COD (72%). In the case of fungi, *A. niger* BOD5 (95%) and COD (89%), and *Penicillium sp*. BOD_5 (83%) and COD (83%), produced results comparable to the work of Paris and Blondeau (1998), which used *Arthobacter* sp. to reduce BOD_5 (80%). Furthermore, Buzzini *et al*. (2005) used prokaryotes from the family *Archae* to reduce BOD_5 (84%), and Tsang *et al*. (2007) used *Neocardia amarae* to remove 94% of BOD_5. Nagarathnama and Bajpai (1999) successfully treated kraft mill effluent using *Rhizopus oryzae*, which removed 50% of COD.

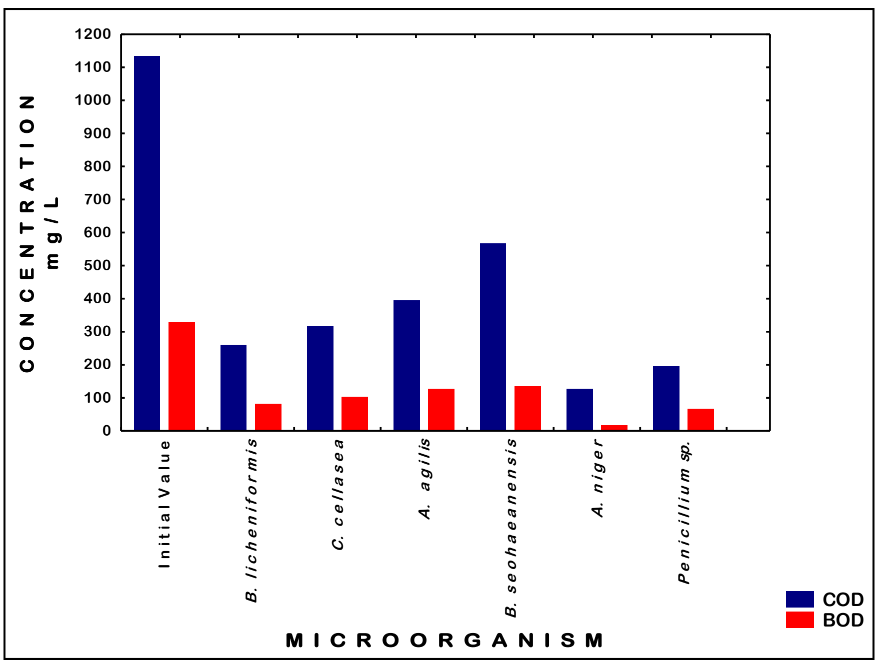

Fig. 2. Decrease in COD and BOD_5 quantities using the selected bacteria and fungi

To determine the efficiency of the treatment, isolated combinations for the formulations based on the behavior of the physicochemical parameters were performed, with the following results.

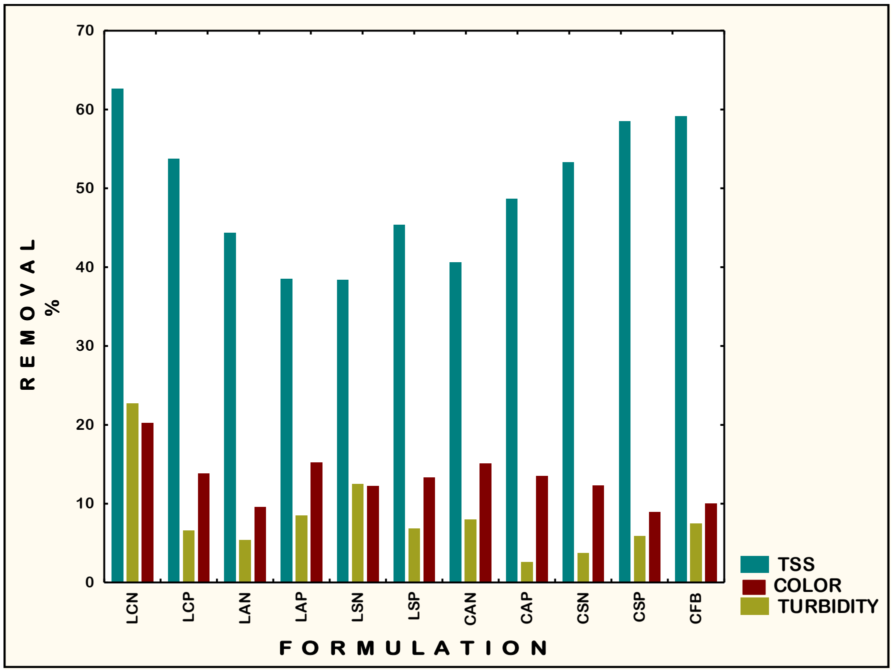

Fig. 3. Removal of parameters turbidity, color, and TSS

In Fig. 3, the reduction of the parameters such as turbidity, color, and TSS are shown using the formulations selected. It is worth noting that the formulations LCN (*B. licheniformis* + *C. cellasea* + *A. niger*), LCP (*B. licheniformis* + *C. cellasea* + *Penicillium* sp.), CSP (*C. cellasea* + *B. seohaeanensis* + *Penicillium* sp.), and CFB (commercial formulation bacterial of Bio-PAPPEL) achieved the best removal rates; however, they collectively failed to reach the limits allowed by CONAGUA (2013). Figure 3 shows a high level of biodegradable organic matter in the effluent (due to the reduction of BOD) which would lead to the depletion of oxygen each time the treated effluent is discharged into a body of water. This invariably can cause the extinction of some aquatic species.

In Fig. 4, the analysis shows a reduction of BOD_5 after biodegradation in all tests compared with the initial value of Table 2. Excellent results were obtained from the formulation of LCN (*B. licheniformis* + *C. cellasea* + *A. niger*), with a BOD_5 of 86% and COD of 84%, LCP (*B. licheniformis* + *C. cellasea* + *Penicillium sp.*) a BOD_5 of 71% and COD of 81%, LAN (*B. licheniformis* + *A. agilis* +*A. niger*) a BOD_5 of 65% and COD of 78%, and CFB (commercial formulation bacterial of Bio-PAPPEL) a BOD_5 of 78% and COD of 82% when compared to the research by Saraswathi and Saseetharan (2010). In the study by Saraswathi and Saseetharan (2010), a formulation of *Pseudomonas alkaligenes* + *T. reesi* + *B. pumilus* was able to remove a BOD_5 of 90% and a formulation of *Pseudomonas alkaligenes* + *B. pumilus* + *B. subtilis* a BOD_5 of 79%, where applicable. Individual strains of the formulations may achieve the best removal results because of "antagonism" or confrontation among the microorganisms when competing for substrate. This happened with Saraswathi and Saseetharan (2010) who tested a strain of *Pseudomonas alkaligenes* (92%) and *Trichoderma reesei* (99%) for BOD_5 removal. The formulation created in this study, LCN, performed better than the commercial formulation of CFB; however, more tests will need to be conducted with as many cells.

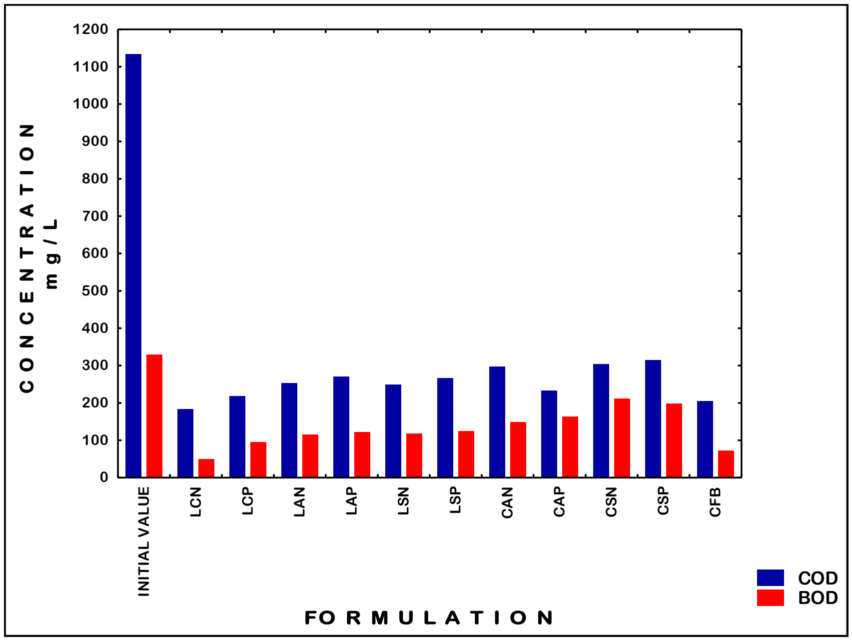

Fig. 4. Decrease in COD and BOD_5 quantities using formulations

Microorganisms are crucial for the biodegradation of pollutants. On the basis of the results obtained, further selections were carried out on the ability of the screened consortia to biodegrade residual of pulp and paper mill effluent. The BOD was applied to new experiments with the consortiums. Pulp and paper mill effluents are characterized by high COD and BOD levels. Webster *et al.* (1997) have reported the inoculation of combined microorganisms, in which the degradation species was the survivor in the effluent treatment. If the native formulation is compared with the commercial formulation, and the native is the best, this indicates that the mixed culture is superior (Hurst *et al.* 1997).

CONCLUSIONS

1. The individual strains of *B. licheniformis*, *C. cellasea*, *A. niger*, and *Penicillium* sp., proved to be excellent degraders of pulp and paper effluent.
2. *A. niger* exhibited the highest degradation activity in the pulp and paper waste when used individually, with a BOD_5 of 95% and a COD of 89%.
3. As a result of this study, the innovative formulation LCN (*B. licheniformis* + *C. cellasea* + *A. niger*) is recommended, as it exhibited the best reduction of BOD.
4. A bacterial formulation that was acclimated to the effluent to be treated outperformed a commercial formulation.

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