Enhancement of domestic wastewater treatment under long sewer line condition in a laboratory set-up by *Aspergillus niger* bioaugmentation

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ABSTRACT

The effect of *Aspergillus niger* bioaugmentation on COD and protein removal in domestic wastewater under sewage conditions was assessed. The sewer simulating bioreactor was running at a hydraulic retention time of 17 h, 20°C and pH 7.8 under aerobic condition. When *A. niger* was bioaugmented, 45 % to 72 % of COD was removed compared to 28 % to 48 % removal of COD in the control at the same period. An overall protein removal of 66 % resulted when *A. niger* was bioaugmented compared to 29.7 % in the control. Regarding enzymatic activities, we observed that as long as the bioaugmented system biomass concentration was higher than the control, the enzymatic activities were also higher. This research is an initial investigation on wastewater transformation under transitory conditions by *A. niger* and demonstrated the capacity of *A. niger* to remove both COD and protein under actual conditions. *A. niger* bioaugmentation under sewer conditions could be an alternative for wastewater treatment with a valorisation of fungal waste biomass.

Key words-*Aspergillus niger*, sewer, wastewater, enzyme, API ZYM.

Nomenclature

COD: Chemical oxygen demand

SEWAP: Sewer wastewater pre-treatment system

LLD: Laser level detector

HRT: Hydraulic Retention Time

INTRODUCTION

Nowadays, wastewater treatment acquires substantial funds of countries and this is viewed to heighten in the future. In developing countries, water resource pollution will reach the critical level if alternative processes are not developed and adapted to actual scenario in treating wastewater. Wastewater treatment in sewer line by bioaugmentation with well-selected microorganisms or development of sewer biofilm could be alternative technologies to prevent this problem (Koch and Zandi, 1973; Green *et al.*, 1985; Hvitved-Jacobsen *et al.*, 1998; Warith *et al.*, 1998; Coulibaly *et al.*, 2002; Coulibaly and Agathos, 2003). Green *et al.* (1985) have already proposed bioaugmentation of indigenous bacteria; but it has not been performed and developed. One of the explanations for this lack of interest could be the high concentration of required mixed liquor. *Aspergillus* and *Trichoderma* are widely used in industries to produce enzymes. The residual biomasses of these fermentations are often stocked in landfill. Otherwise, as it is well known, active fungal biomass of *Aspergillus* has relevant potential in pollutant degradation (Coulibaly *et al.*, 2003). We have used *A. niger* under transitory condition to degrade starch as a model polysaccharide substrate and a mixture of this substrate and bovine serum albumin (Coulibaly *et al.*, 2002; Coulibaly and Agathos, 2003). The fungus was capable of transforming biopolymers to easily degradable substrates with simultaneous secretion of enzymes in its growth medium.

The aims of this research were (i) to assess the ability of *A. niger* in removing chemical oxygen demand (COD) and proteins in raw wastewater under long sewer line condition at hydraulic retention time (HRT) of 17 h (Ozer and Kasirga, 1995), (ii) to investigate the valorisation in wastewater treatment field of waste biomass of *A. niger* and (iii) to verify the secretion of extracellular enzymes. The strain *A. niger* was chosen after screening of several strains of *Trichoderma* and *Aspergillus* for their capacity to excrete hydrolases while growing in domestic wastewater. The fungal biomass was simply produced in the laboratory and a sewer simulating bioreactor was designed to perform the biodegradation test.

METHODS

Microorganisms and culture conditions

Aspergillus niger. MUCL 28817 was obtained from the fungal collection of the Catholic University of Louvain (MUCL). The fungus was cultivated on tryptic soy agar (TSA) from Difco laboratories (Detroit, Mich., USA) in 260 ml flat bottle (Nunc, Roskilde, Denmark) containing 40 ml of TSA at 28°C for 7 days prior to usage in the reactor system. *A. niger* was precultured in the medium described by Garcia *et al.* (1997) containing 40 g l⁻¹ glucose, 5 g l⁻¹ meal peptone and 5 g l⁻¹ casein peptone. Two millilitres of fungal spores collected from 2 bottles of TSA with 20 ml of preculture medium containing 0.1% Tween 80 were inoculated in a 250 ml baffled flask containing 100 ml of preculture medium. The fungus was grown for 3 days at 20°C on a rotary shaker at 150 rpm. After 3 days, the biomass was recovered by vacuum filtration under sterile conditions on a Whatman N°4 filter paper. The biomass was suspended and washed in sterile water. The filtration and washing steps were repeated three times. After the third filtration, the recovered fungal biomass was suspended in a 250 ml flask containing 100 ml of sterile water. The biomass was homogenised for 5 min with a tissue homogeniser (Ultra-Turrax, Stanfen, Germany). An aliquot of 50 ml was washed and recovered as above and the biomass was used to inoculate the reactor.

Reactor system. The sewer simulating system used in this research had been previously described in Coulibaly *et al.* (2002). It was composed of five stirred tanks in series. The system included one membrane pump (Prominent, CfG, Heidelberg, Germany), which fed the first reactor, and four peristaltic pumps (Gilson, Manupilus 2, Namur, Belgium) linking each reactor to its neighbouring unit. The reactors and the feeding reservoir were agitated with magnetic stirrers (Ika-Combimag RCO, Namur, Belgium). The reactor system was operated at an overall hydraulic residence time (HRT) of 14 h, which is encountered in long sewer lines (Özer and Kasirga, 1995).

Reactor inoculation and sampling. The reactor system was inoculated and sampled in the same way as described in our previous research (Coulibaly *et al.*, 2002). It was filled with 500 ml of raw wastewater. The first reactor was then inoculated with biomass prepared as indicated. The sampling period determination has been described in previous research (Coulibaly *et al.*, 2002).

Wastewater. Wastewater was taken from a Louvain-la-Neuve collector. The raw wastewater was sifted in situ on a 500 µm sieve and taken to feed the SEWAP. Approximately 30 litres were preserved at 4°C no more than a week for the control, to avoid variation in wastewater characteristics.

Analyses. Fungal biomass was determined by dry cell weight (oven drying at 105°C for 24 h) and COD was determined by the dichromate method NBN-T 91-201. Proteins were determined by multiplying the organic nitrogen portion by 6.25 (Sridhar and Pillai, 1973). The organic nitrogen portion was determined by the difference between the total nitrogen (Kjeldahl method, NBN-T 91-255) and ammonium (NBN-T-91 255). Enzymatic profiles, on the other hand, were determined with the API ZYM kit from bioMerieux (Marcy-l'Etoile, France) and the manufacturer's instructions were followed throughout. The API ZYM kit is a standardised semi quantitative micro method able to detect 19 different types of enzymes. It has previously been used to screen enzyme profile in environmental research (McKellar, 1986; Boczar *et al.*, 1992; Morgan and Pickup, 1993; Cicek *et al.*, 1998).

RESULTS AND DISCUSSION

Experiments were performed at a hydraulic retention time (HRT) of 17 hours, pH 7.8 and 20°C. Figure 1 shows the kinetics of COD removal in the sub-reactors R₁, R₃ and R₅. The kinetics for sub-reactors R₂ and R₄ are not reported because of the similarities between R₂ and R₁, and between R₄ and R₅. One could observe that COD removal increased from reactor R₁ to R₅ (figure 1) in both the bioaugmented system and the control. However, for each of the reactors, COD removal in the bioaugmentation experiment was superior than the control. The increase in COD removal parallel with the reactor order could be explained by the augmentation of the contact time between the pollutants and the biomass. In fact, the HRT in R₁, R₃ and R₅ were respectively 3.4, 10.2 and 17 h. In R₅ (figure 1. C), 70% of COD and about 66% of proteins (table 1) were removed during the bioaugmented process while at the same time and within the same reactor in the control, only 48% of COD and 30% of proteins (table 1) were removed. The remaining protein concentrations in the effluents of the bioaugmented reactor and the control were respectively, 22 mg l⁻¹ and 48 mg l⁻¹ (table 1). The equivalent COD of these proteins are 35.2 mg O₂ l⁻¹ and 76.8 mg O₂ l⁻¹, respectively, in the effluent of the bioaugmented reactor and in the control.

Figure 1. Kinetics of soluble COD removal in domestic wastewater by *A. niger* and the control under transitory conditions. In reactor R₁ (A), R₃ (B) and R₅ (C). Bioaugmentation with *A. niger* (—■— COD, —▲— Biomass) Control (—▲— COD, —□— Biomass)

One could observe that proteins constituted the essential of the remaining COD in the effluent of reactor R₅. The higher concentration of proteins observed in the effluents of reactors could be explained by their low biodegradability and the decrease in activities of proteases (Lotter and Van der Merwe, 1987; Coulibaly and Agathos, 2003).

The kinetic in terms of COD uptake rate in the sub-system (R₁ to R₃; HRT = 10.2 h) could be compared with the experiment of Green *et al.* (1985) performed on Tel-Aviv sewerage system (HRT = 9.8 h). When *A. niger* was bioaugmented, the initial biomass in the subsystem (R₁ to R₃) was 503 mg l⁻¹. At this biomass concentration, the COD uptake rate was 1.84 g COD (g SS)⁻¹ at 31 h while it was 0.43 g COD (g SS)⁻¹ when Green *et al.* (1985) bioaugmented 538 mg l⁻¹ of activated sludge. One could observe that *A. niger* bioaugmentation enhanced the COD uptake rate four times than activated sludge bioaugmentation.

Table 1. Kinetics of protein removal in domestic wastewater by *A. niger* and the control under transient conditions.

Time (h)	Control Run			Bioaugmentation Run		
	Feed (Protein, mg l ⁻¹)	Effluent (Protein, mg l ⁻¹)	Removed (%)	Feed (Protein, mg l ⁻¹)	Effluent (Protein, mg l ⁻¹)	Removed (%)
0	67.5			65		
7	67.5	57.5	14.8	75	47.5	26.9
14	67.5	37	45.2	75	48.7	35.1
24	63.7	55	13.6	75	52.5	30
31	63.7	39.4	38.1	75	31	58.7
38	87	48	44.8	75	22	70.7

Biodegradation mechanism underlying the bioaugmented reactor system was further characterised by analysing enzymatic profiles in the wastewater feed and in R₁, R₃ and R₅ effluents using API ZYM kit at 7 h, 14 h and 31 h, respectively (figure 2).

Figure 2. Enzymatic profiles in wastewater under transitory conditions. In feed (A), R₁ (B), R₃ (C) and R₅ (D). Bioaugmentation with *A. niger* (▨), Control (□)

These periods were selected in order to verify if fungal biomass had an effect on the activities of enzymes in their growth medium. One could observe that at 7, 14 and 31 h, the suspended solid concentration in the bioaugmented reactor was respectively, superior, equal and inferior to the control (figure 1). The conservation of wastewater at 4°C influenced the enzymatic profiles (figure 2 A). The main enzymes present in the wastewater feed (figure 2 A) were phosphatase (alkaline, acid and phosphohydrolase), cellulase (α -glucosidase, β -galactosidase, α -mannosidase), esterase (C4 and lipase C8) and protease (leucine aminopeptidase). The enzymatic activities of the wastewater conserved at 4°C were generally superior to that which was not conserved at this temperature. The enzymes characterized in this research are specific to wastewater (Hankin and Sands, 1974; Verstraete *et al.*, 1976; Lotter and Van der Merwe, 1987; Nybroe *et al.*, 1992; Lemmer *et al.*, 1994) and may not have only bacterial origin, but also human excreta origin (Nybroe *et al.*, 1992). The highest activity of alkaline phosphatase at the beginning of the experiment could have originated from human excreta (Verstraete *et al.*, 1976). They could have some effects of the fungus upon enzyme production in the growth medium (figure 2 BCD). One could remark that, when the biomass concentrations of the bioaugmented system were superior or equal to the control biomass (7 h in R₁ and 14 in R₃), the enzymatic activities in the former system were essentially superior to the latter. On the other hand, when the biomass concentrations in the bioaugmented system in a sub-reactor were inferior to the control biomass, the enzymatic activities were essentially inversed. The higher phosphatase activity observed at 38 h for the control in R₅ could be the feed activity brought by dilution.

CONCLUSION

This research demonstrated the possibility to enhance soluble COD and protein removal by augmentation of wastewater with *A. niger* in a sewer simulating system running at a HRT of 17 h. COD and proteins were removed twice higher in the bioaugmentation reactor system than in the control. The findings of this research bring out the *A. niger* applicability in complex media such as sewage. Bioaugmentation of sewage with *A. niger* under transitory condition could be considered for wastewater pretreatment as there is considerably less biomass inoculated. Further investigations are necessary to assess both nutrient removal and bioaugmentation process modelling in order to give an accurate tool for sanitary engineers. This could be more economical for wastewater treatment as well as for the management of *A. niger* waste biomass originating from fermentation industries.

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