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Full Length Research Paper

Kinetics study of bioremediation of industrial effluents by *Pseudomonas spp* and *Bacillus spp* using chemical oxygen demand (COD)

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The kinetics study of effluents from food and detergent industries were carried out. Two different bacteria *Pseudomonas spp* and *Bacillus spp* were isolated from food effluent (FE) and detergent effluents (DE) and were used for their bioremediation respectively. Reductions in the chemical oxygen demand (COD) of the effluents were studied as indication of bioremediation of the effluents. The overall result showed that the reduction in the chemical oxygen demand (COD) for the food effluent and detergent effluent at 5g of micro-organism (OW5g) was 78.70% and 84.47% respectively while those of 10g of micro-organism (OW10g) were 91.11% and 87.76% respectively. At 50% dilution of the effluents and 5g of the micro-organism (DW5g) the reduction in COD was 96.83% and 88.73% for food effluent and for detergent effluent respectively. The reductions in COD were subjected to zero and first order kinetics. The rate constant for COD reduction at 5g of micro-organism for zero order and first order kinetics were (COD-F 33.8mg/l/d and 0.061d^{-1}) and (COD-D 31.7 mg/l/d and 0.067d^{-1}) respectively. The rate constants for COD reduction at 10g of micro-organism for zero order and first order kinetics were (COD-F 31.09mg/l/d and 0.123d^{-1}) and (COD-D 32.4mg/l/d and 0.085d^{-1}), while those at 50% dilution with 5g of micro-organism was (COD-F 30.8mg/l/d and 0.786d^{-1}) and (COD-D 28.45mg/l/d and 0.14d^{-1}). The kinetic models results showed that both the COD reduction for *pseudomonas spp*. and *bacillus spp*. used in this research followed both the zero order and first order kinetics. However, while zero order kinetics was best fitted for the original sample with 5g of immobilized microorganisms, first order kinetics was well fitted when the concentration of both effluents was reduced by 50% with 5g immobilized microorganisms and original sample with 10g of the immobilized microorganisms.

Key words: Kinetics, chemical oxygen demand (COD), immobilization, food effluent (FE), detergent effluent (DE)

INTRODUCTION

Bioremediation aimed at the reduction of contamination in a matrix, often based on the introduction of inorganic nutrients for enhancing the performance (Leahy and Colwell, 1990).

Bio-treatment is assessed by increase in biomass growth and reduction in the substrate. *Bacillus subtilis* and *Micrococcus luteus* have been found to have high biomass growth and substrate reduction among group of bacteria isolated from petroleum contaminated wastewater (Hamza *et al.,* 2008).

Chemical oxygen demand (COD) which measures the oxygen equivalence of the constituents in the wastewater is used as a measure of substrate composition (Tchobanoglous *et al.,* 2003). The COD and BOD are gross overall indicators of sewage composition and they therefore do provide a measure which relates to the

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potential environmental damage of a wastewater. Though, COD has advantage over BOD of being measurable in about 2 hours by conventional methods or in a few minutes using sophisticated instruments (Bailey and Ollis, 1986), if large amount of chemicals enter wastewater chemical reaction occur which consume large amount of oxygen (i.e. high COD). On the other hand, if the oxygen level of water drops too much fish and other aquatic life may not survive (Wisconsin department of natural resources, 2006).

A variety of microbial growth and biodegradation kinetics models have been used for removing both organic and inorganic materials from aqueous solution. Such model allows the prediction of amount of chemicals that remain at a given time and the calculation of the time required to reduce chemicals to a certain concentration (Okpokwasili and Nweke, 2005). Knowledge of bio-kinetics is also essential for biological wastewater system design and optimization of operational conditions (Nakhla *et al.,* 2005). Many industrial wastewaters, including food processing wastes, invariably contain colloidal and particulate organics that undergo hydrolysis prior to biodegradation. Morgenroth *et al*. (2002) have reviewed the kinetic modelling of hydrolysis in municipal wastewater treatment and noted that the most widely used kinetic model was first order with respect to particulate substrate.

Immobilized cells have been used and studied extensively for the production of useful chemical (Ohta *et al*., 1994; Chang and Chou, 2002), the treatment of wastewaters (Chen *et al*., 2000; Wang *et al*., 2000), and the bioremediation of contamination not only simplifies separation and recovery of the immobilized bacteria and the binding agent, but it also makes the application reusable, which reduces the overall cost. Immobilized materials, furthermore, have comparatively longer operating lifetimes due to an enhanced stability of the macromolecules or cells and consequently, protection from adverse conditions. (Diaz *et al*. 2002).

First-order kinetics is commonly used to describe biodegradation in environmental fate models because mathematically the expression can be incorporated easily into the models (Greene *et al*. 2000). Many investigators grasp at first-order kinetics because of the ease of presenting and analysing the data, the simplicity of plotting the logarithm of the chemical remaining versus time as a straight line, and the ease of predicting future concentrations (Hutchins *et al*. 1991; Greene *et al*. 2000; Reardon *et al*. 2002).

Though COD and BOD have been widely used as the overall indicators of sewage composition and they therefore do provide a measure which relates to the potential environmental damage of a wastewater, data on the rate at which this is achieved in the treatment of waste from specific industries have remained scanty or not being documented at all. This work therefore aimed at obtaining the kinetics dataforthe COD removal using immobilized bacteria isolated microorganism from food and detergent effluent.

MATERIALS AND METHODS

Sampling area and collection

Food and detergent effluents were collected from the effluent tanks of NASCO group of company in a sterile 4 litres plastic containers carried in an ice-chest and stored in a refrigerator before analysis. The sample was collected from the channel to point of discharge (Adebayo *et al*., 2010).

Microbiological analysis of the effluent.

A sterile Nutrient Agar plate was inoculated with 0.1ml dilution of the effluent using the Pour plate method and incubated upside down at 35°C for 24hrs for the bacteria isolation. This was followed by the total microorganism count multiplied by the dilution factor. Characterization and Identification of Isolated were carried out as described by (Fawole and Oso, 1988). The microorganism of interest which outgrows other species was singly inoculated as the predominant specie and was used in each case for this research.

Agar-agar medium was used for the immobilization of the microorganisms using Ellaiah *et al*. (2005) method. The solidified block was cut into cubes.200.00ml of sterile effluent samples were taken into sterile 250ml conical flasks and 5.0g of corresponding immobilized bacteria samples were transferred into each effluent. These samples were then taken into a temperature controlled rotating water bath for agitation. 10ml of samples were taken for C.O.D. determination on daily basis for five days. The process was repeated with 10g of immobilized bacteria samples and also by diluting the effluents with 50% distilled water and 5.0g of immobilized bacteria.

Determination of COD

The chemical oxygen demand of the effluent was analysed before and on daily basis for a period of 5 days treatment of the samples with isolated microorganisms as described by (Nsi, 2007), (APHA standard method, 1989) and (Ademoroti, 1989).

RESULTS AND DISCUSSIONS

The substrate consumption was indicated by the reduction in chemical oxygen demand (COD). The overall COD reduction was from 188 to 4mg/l and 206 to16 mg/l by *Pseudomonas spp*. and *Bacillus spp.* on food and

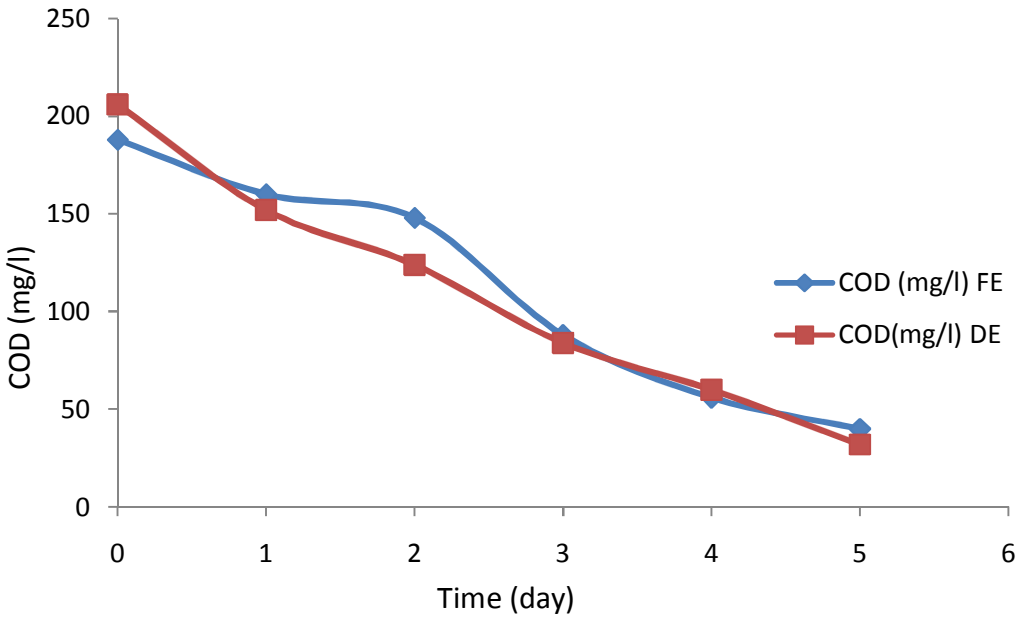


Figure 1. COD removal trend (Original sample with 5g of micro-organism).

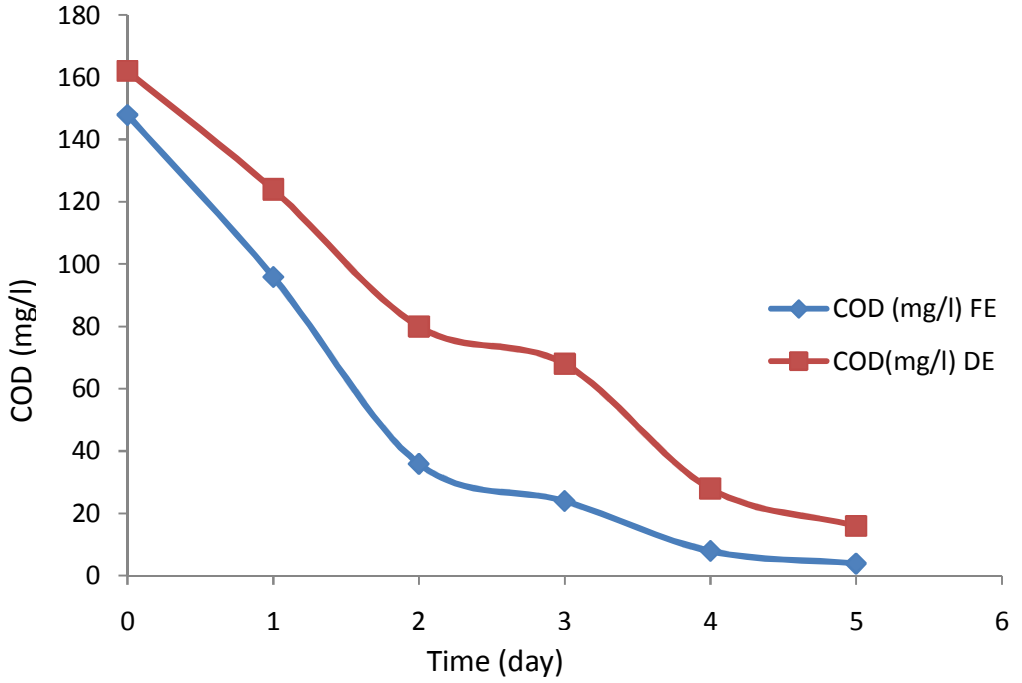


Figure 2. COD removal trend (50% diluted sample with 5g of micro-organism).

detergent effluents respectively. It is obvious that the rate of COD removal is divided into rapid removal and moderate removal stage (Guan *et al*., 2003). The results showed that the two bacteria are capable of reducing the COD from food and detergent effluents. The kinetics of the COD reduction was determined by fitting the COD data through different kinetic models. The data fitted into zero and first order kinetic models as shown in Figures 1 to 9.

After five days of biochemical reactions, there was a significant reduction in the values of COD of both the food effluent and detergent effluent. The overall result

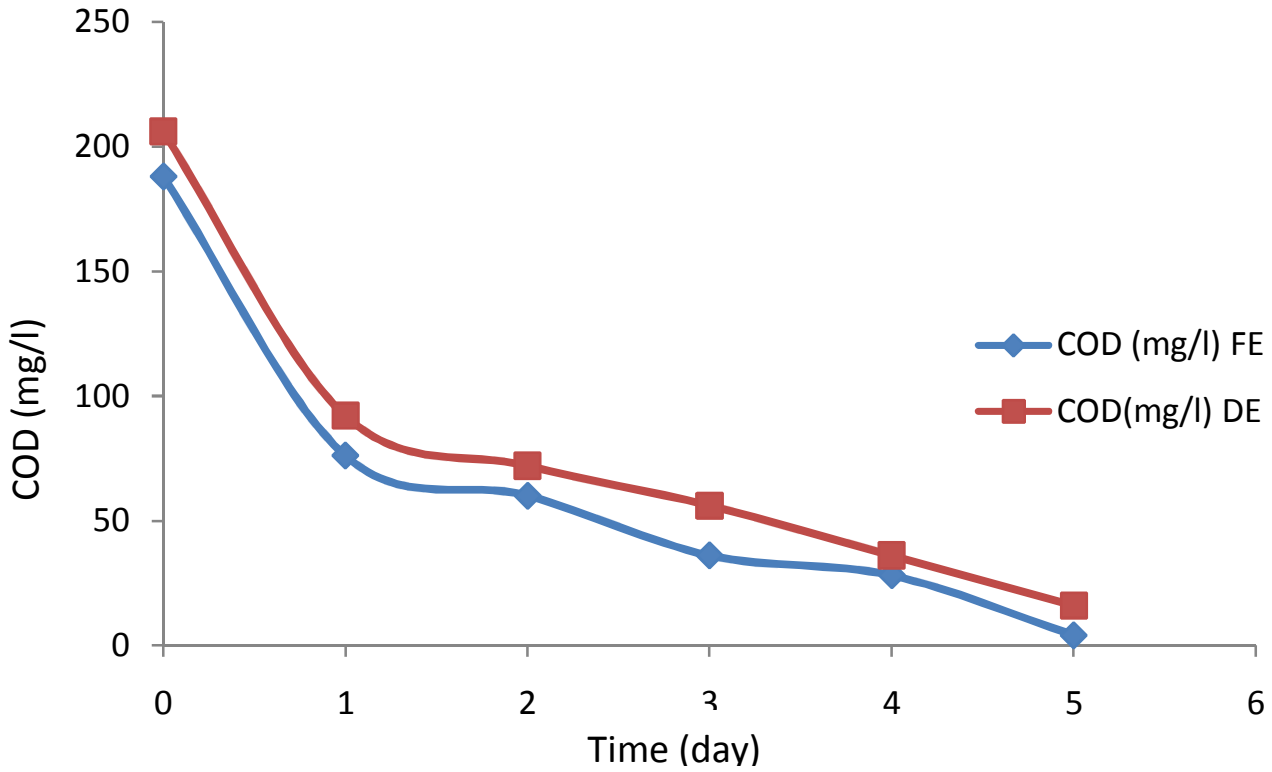


Figure 3. COD removal trend (Original sample with 10g of micro-organism).

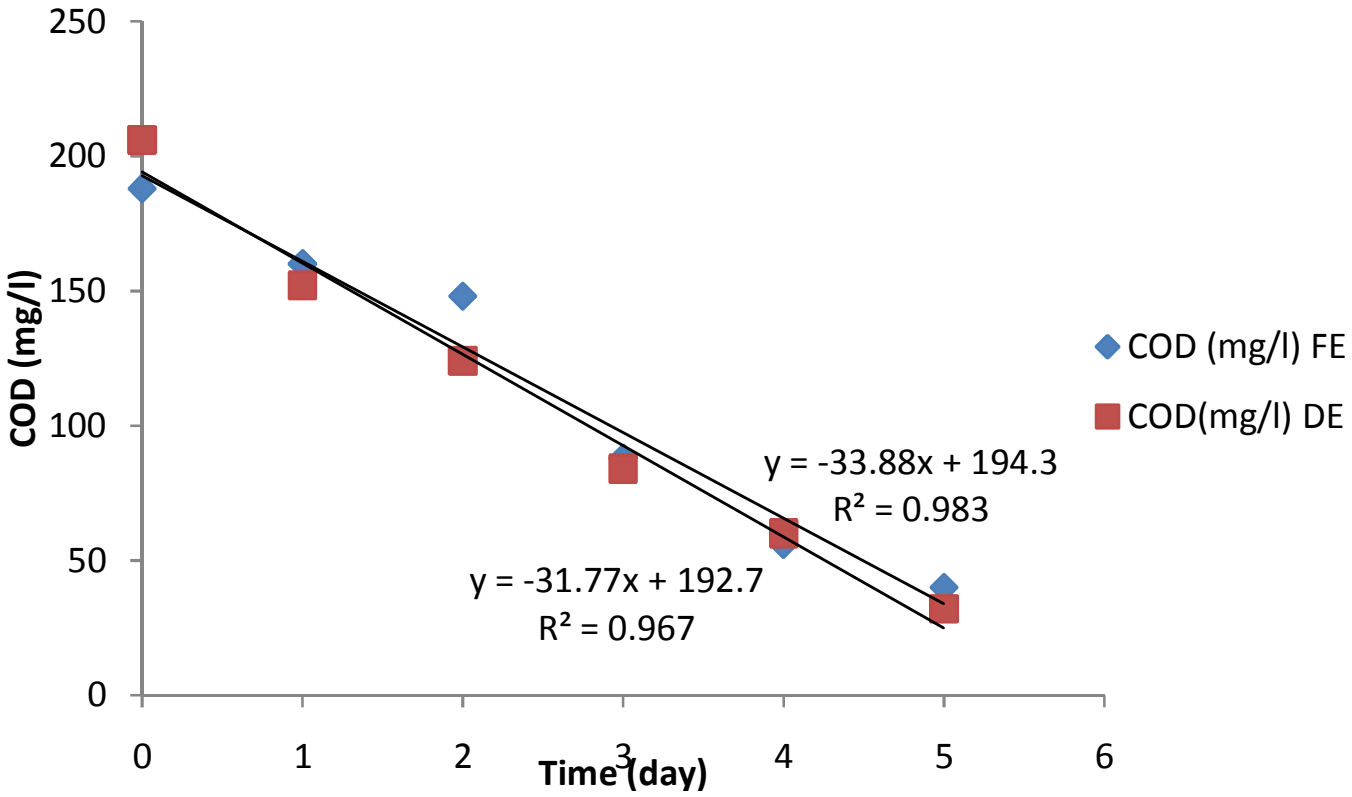


Figure 4. Testing Zero Order Kinetics for COD (Original sample with 5g of micro-organism).

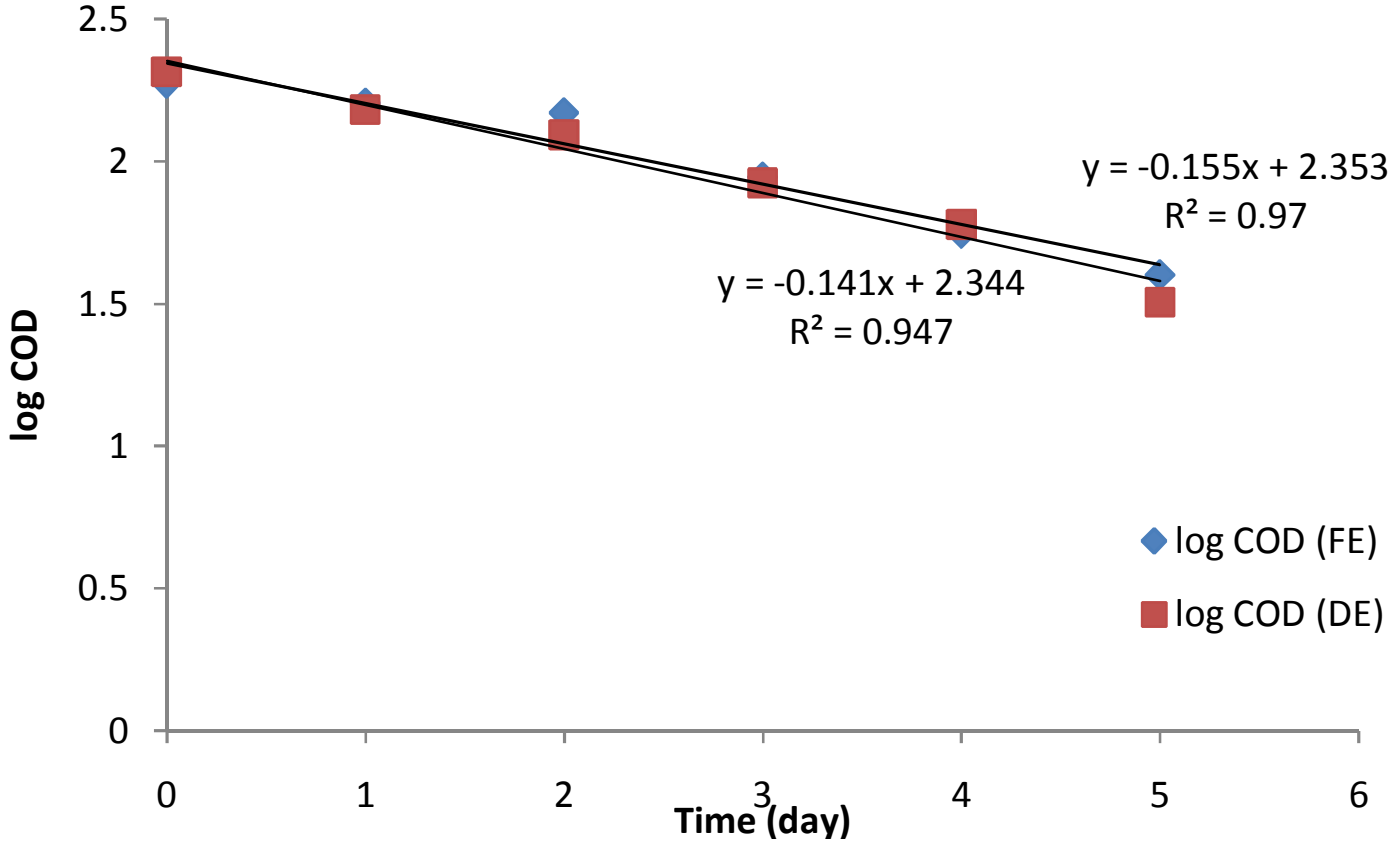


Figure 5. Testing First Order Kinetics for COD (Original sample with 5g of micro-organism).

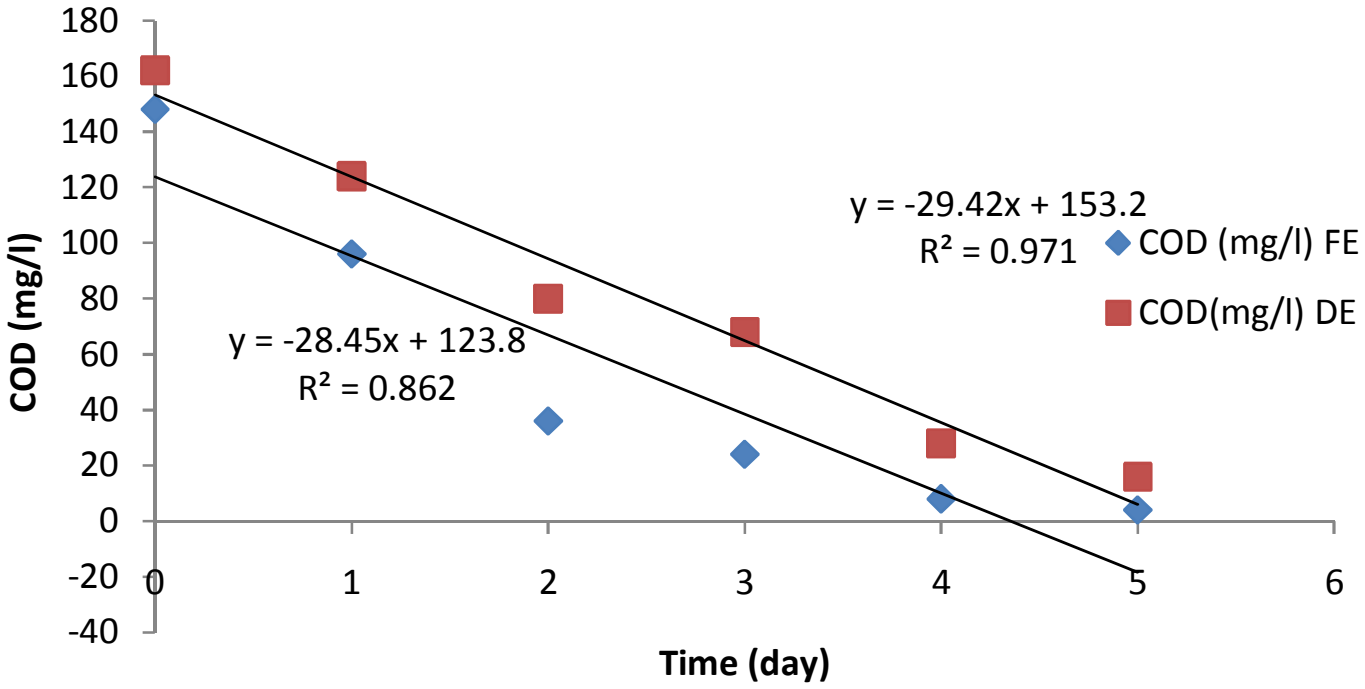


Figure 6. Testing Zero Order Kinetics for COD (50% Diluted sample with 5g of micro-organism).

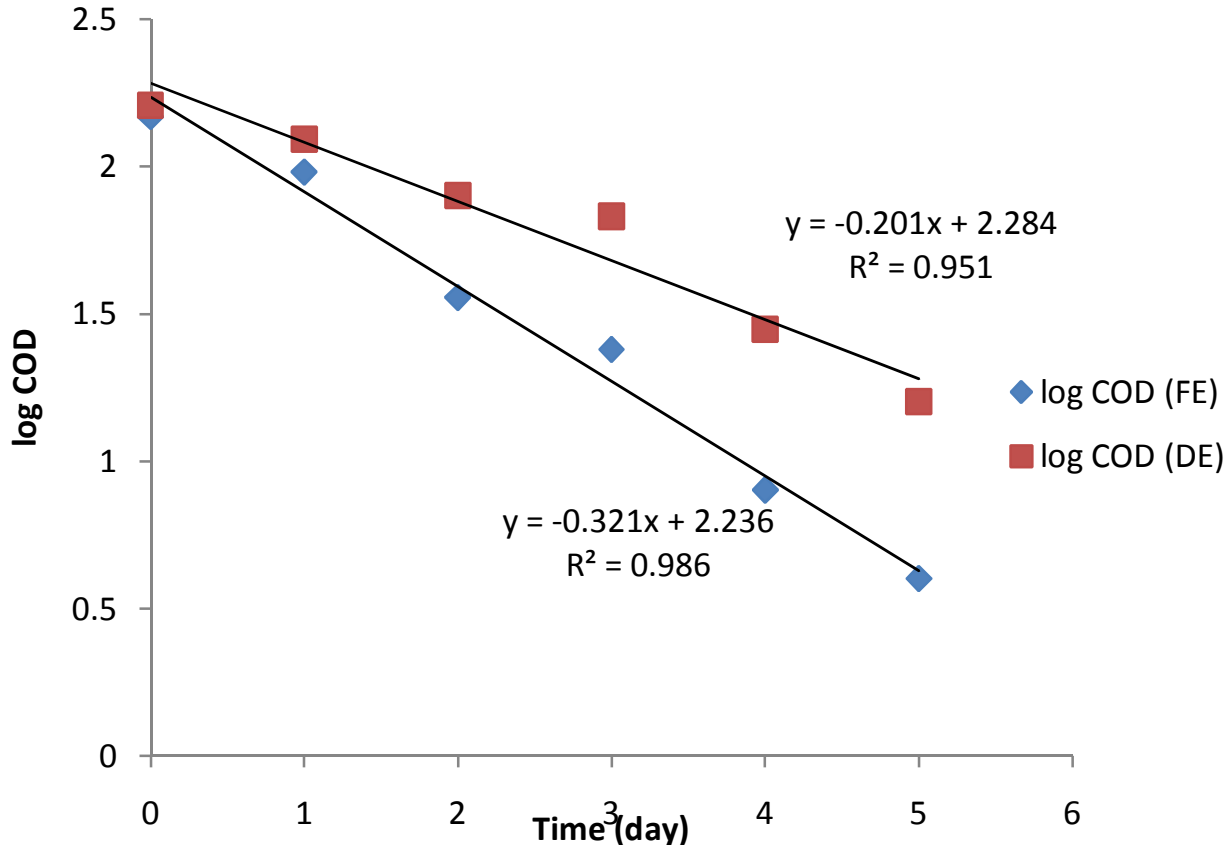


Figure 7. Testing First Order Kinetics for COD (50% Diluted sample with 5g of micro-organism).

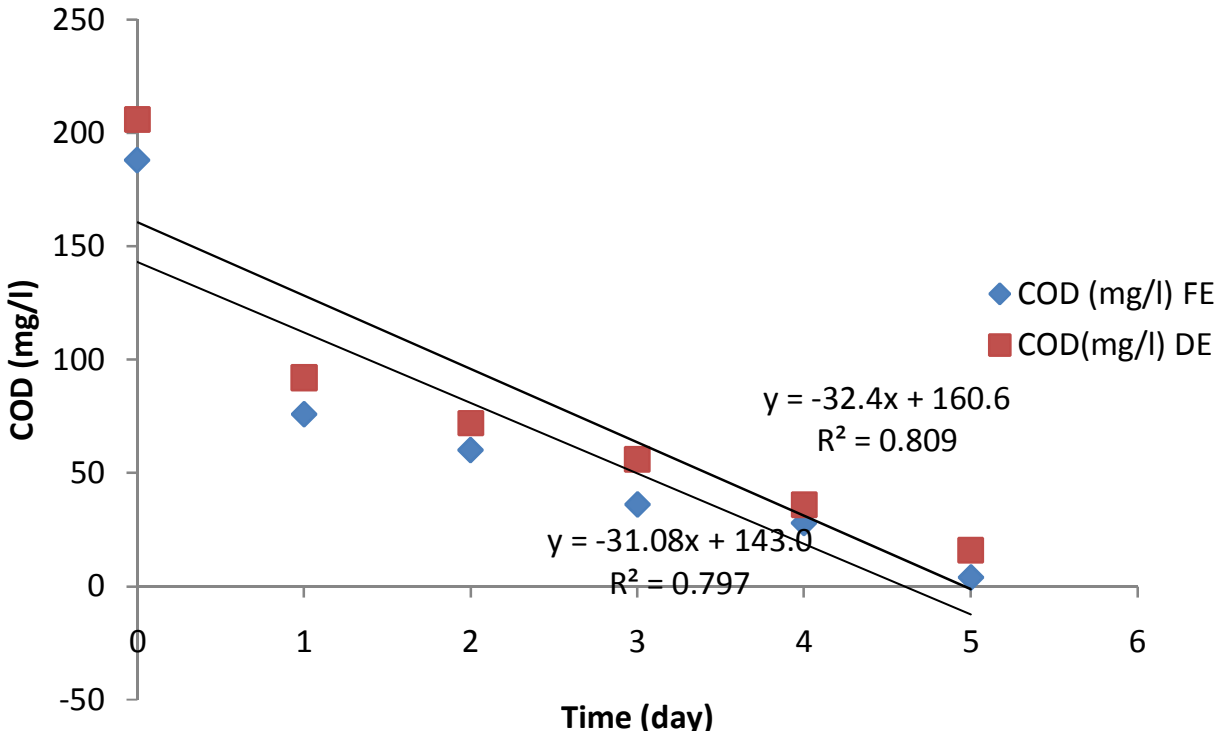


Figure 8. Testing Zero Order Kinetics for COD (Original sample with 10g of micro-organism).

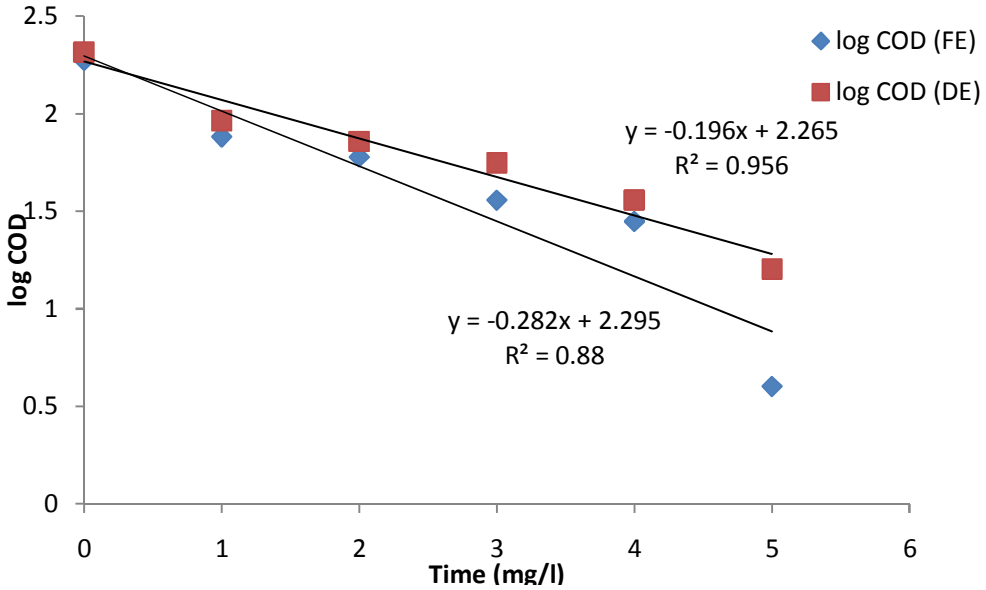


Figure 9. Testing First Order Kinetics for COD (Original sample with 10g of micro-organism).

Table 1. Reduction of COD for FE and DE.

Day	COD(mg/l) OW5g		COD(mg/l) DW5g		COD(mg/l) OW10g	
	FE	DE	FE	DE	FE	DE
0	188	206	148	162	188	206
1	160	152	96	124	76	92
2	148	124	36	80	60	72
3	88	84	24	68	36	56
4	56	60	8	28	28	36
5	40	32	4	16	4	16

Table 2. Summary of thekinetic constants of the COD reduction.

Parameters	Kinetics constant	
	Zero (mg/l/d)	First (d^{-1})
COD (F) original sample with 5g microbial	33.80	0.061
COD (D) original sample with 5g microbial	31.70	0.067
COD (F)50% dilution with 5g microbial	30.80	0.14
COD (D) 50% dilution with 5g microbial	28.45	0.087
COD (F) original sample with 10g microbial	31.09	0.123
COD (D) original sample with 10g microbial	32.40	0.085

showed that the reduction in the chemical oxygen demand (COD) for the food effluent treated with 5g of micro-organism was 78.70% and that of the detergent effluent 84.47% while those treated with of 10g of micro-organism was 91.11% for food effluent and 87.76% for detergent effluent. Similarly, the reduction of COD with 50% dilution of the effluents treated with 5g of the micro-organism gave 96.83% for food effluent and 88.73% for detergent effluent (Table 1).

The reductions in COD were subjected to zero and first order kinetics. The overall rate of removal of COD *by pseudomonas spp*. and *bacillus spp*. in food effluent and detergent effluent respectively follow both the zero and first order kinetics. The summary ofthe kinetics rate constants and initial COD concentration/metal concentration, Co were shown in Table 2. These values were obtained

from the above plots of the integrated rate expression for zero order (Equation 1) and for first order (Equation 2).

$$[COD]_t = [COD]_0 - kt \ldots\ldots\ldots\ldots 1$$

$$\log[COD]_t = -2.303\ kt + log[COD]_0 \ldots\ldots\ldots 2$$

It can be observed that the rate of COD removal for both food and detergent effluents were zero order at a high COD values. This is because at high concentration of COD, every site of the organism is saturated with the substrate which made the rate to be constant (i.e. zero order).

However, at low COD values it was observed that the rate was 1st order kineticsindicating that, as the COD concentration decreased only few available site of the organism was covered and made the rate of reaction to be proportional to the substrate concentration i.e. first order as previously reported (Waterloo, 2006).

Conclusion

It has been observed from the results obtained that *Pseudomonas spp.* and *Bacillus spp.* have high ability to reduce chemical oxygen demand from Industrial effluents such as those of food and detergent respectively. The reduction in the chemical oxygen demand (COD) for the food effluent at 5g of micro-organism was 78.70% and that of the detergent effluent 84.47% while those of 10g of micro-organism was 91.11% for food effluent and 87.76% for detergent effluent. Also the reduction of COD with 50% dilution of the effluents and 5g of the micro-organism gave 96.83% for food effluent and 88.73% for detergent effluent. The kinetics of the COD reduction obeys zero order kinetics at high substrate concentration and first order at low substrate concentration.

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