Aerobic Bioremediation of Fischer-Tropsch Effluent – Short Chain Alcohols and Volatile Fatty Acids

Mabatho Moreroa, Diane Hildebrandt and Tonderayi Matambo

***Abstract*— Aerobic degradation was used as a treatment method to reduce the high chemical oxygen demand (COD) found in Fischer-Tropsch (FT) wastewater. The compounds investigated were short chain alcohols (SCA) and volatile fatty acids (VFAs), they contribute up to 87.4%. When released into the environment, such high strength COD can cause detrimental effect to the environment. Synthetic FT wastewater were prepared in a mineral salt solution comprising of only SCA's and VFA's and a COD of 67.9 gCOD/L. Parameters investigated were temperature and substrate concentration (COD). Bacteria found in natural wetland situated east of Gauteng province in South Africa and FT wastewater plant, were collected, studied and used in this study. It was observed that degradation was favoured at 35°C with 90% COD removal within 3 days. At substrate concentrations of 0.13, 0.73 and 1.5%, the highest COD reduction was 91, 49 and 24% respectively. The isolates were sent for 16S rRNA sequence analysis. *Bacillus* sp. was found to be dominant.**

***Index Terms*— aerobic degradation, chemical oxygen demand (COD), Fischer-Tropsch effluent.**

I. Introduction

South Africa has been deemed as a water scarce country. With the country classified as semi-arid, there are limited fresh water resources, thus limiting the available water resources [1]. It has been projected that by the year 2025, most countries in the eastern and southern Africa will have limited water availability. Twelve African countries will be limited to about 1000 m^3/person/year of fresh water (thus 2.7 L/person/day), furthermore, 460 million people will be at risk of water stress. In 2018, one of the biggest cities in South Africa, Cape Town, is experiencing the worst drought in 100 years, with the dams supplying the city below 25% [2]. This led to water restrictions of 50 litres per day per person, which was a further decrease from a previous 87 litres per day per person. It was reported that from May 2018, water supply in the city will be cut off and residents will have to visit one of the 200 water collection sites for water collection [3]. The National Water Act, 1998 (Act 36 of 1998) of South Africa requires a balance between using and protecting water resources to be achieved. One of the ways of achieving this, is through reclaiming and reusing treated wastewater which will in turn create an alternative water source for irrigation and other uses of water that do not require clean fresh water. This will assist in reducing the demand for portable water sources utilized for drinking. It has then become necessary to find the biggest contributors to water pollution and tackle the problem from the source. One of the biggest contributors to water pollution has been identified as the petrochemical industry. The products from this industry are good for economic growth, but the pollutants can pose a detrimental effect on the environment. It has been reported that for every ton of crude oil produced from the Fischer-Tropsch (FT) process (a process used in the petrochemical industry), there is 1.1 to 1.3 tons of wastewater produced, which is the largest volume liquid product from the process [4], [5], [6]. Wastewater generated from this process contains high amounts of organic matter (30 g/L COD); which comprises of alcohols (84.8%), acids (10.7%), hydrocarbons (4.50%) and low pH value (pH = 3.0) [7]. Biodegradation of this type of wastewater has been done using anaerobic systems, which require skilled labor to maintain, require lengthy start-up time (2-4 months) and sensitive to temperature changes [8].

Due to cost implications and time constraints, it was then necessary to study the biodegradation of the compounds found in the FT wastewater in an aerobic environment. The results from this study can then be optimized and used to address the problems associated with the treatment of this wastewater in a larger scale. It has been reported that aerobic systems are used to treat wastewater with high organic loads to achieve high degree treatment efficiency and excellent effluent quality [8].

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II. Experimental

A. Preparation of synthetic Fischer-Tropsch wastewater

Synthetic wastewater was prepared in a mineral solution [9] by adding compounds found in FT wastewater as indicated in Table I [9], which served as carbon source (CS).The stock solution, with COD 67.9 g/L, was used to investigate the effect of compound concentration by serial dilutions.

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TABLE I:
PREPARATION OF SYNTHETIC WASTEWATER

	Compound	Volume (ml/L)
Short Chain Alcohols (SCA)	Methanol	3,01
	Ethanol	3,20
	Propanol	2,87
	Butanol	2,12
	Pentanol	1,22
Volatile fatty acids (VFA's)	Acetic acid	0,962
	Propanoic acid	0,696
	Butanoic acid	0,361
	Pentanoic acid	0,105
	Hexanoic acid	0,026

B. *Sample collection*

Sludge was collected from an FT operating plant's aerobic and anaerobic digester and from a natural wetland in the east Johannesburg, using 5.0 L autoclaved schott bottles. The sludges were stored at 4°C and used during this study.

C. *Culturing bacteria*

All apparatus was autoclaved at 121°C and for 20 minutes and media inoculation was carried out in a laminar flow hood. Nutrient broth and agar obtained from Sigma Aldrich were used to prepare agar plates. An inoculation loop was used to transfer the bacterial consortium from the sludges onto the agar plates. The plates were incubated at 35°C overnight for cell multiplication. Conical flasks were prepared with 50 ml mineral solution [9] and inoculum from the agar plates. The flask was incubated at 35°C with shaking 100 rpm for 2 days, to obtain an optical density (OD) reading of 1.0 at wavelength 600 nm.

D. *Effect of substrate concentration*

Conical flasks were prepared from the stock solution and serial dilutions were done to vary the concentration of substrates (0.13, 0.73 and 1.5%) in the mineral solution. An ultra-violet (UV) spectrophotometer supplied by ThermoFisher Scientific (GEN10S UV-Vis) was used to measure the OD of the samples within the media. A thermo reactor supplied by Lovibond® (RD125) was used to digest samples before reading the COD. A COD kit supplied by Lovibond® (MD 600) was used to measure the COD readings during the study. Table II shows the experimental set-up that was used.

Table II
PREPARATION OF EXPERIMENTAL SET-UP

Assay	Media (ml)	Media with CS (ml)	Inoculum (ml)
ISC	50	0	0
SC	50	0	1
IC	50	5	0
Test	50	5	1

ISC: Inoculum and substrate control, SC: Substrate control, IC: Inoculum control, Media: Mineral solution only, CS: Carbon source.

III. RESULTS AND DISCUSSIONS

A. *Effect of substrate concentration*

The stock solution with initial COD of 67.9 g/L was diluted using mineral solution to prepare three concentrations in triplicates i.e. 0.13, 0.73 and 1.5%. Fig. 1 shows the OD of the solutions.

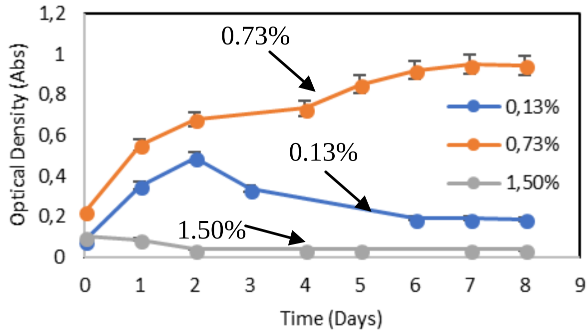

Fig. 1: Effect of substrate concentration on bacterial growth

An increase in biomass was observed in the flasks that contained 0.13 and 0.73% carbon source. The OD in the flask that contained 0.13% substrate, dropped on the third day of incubation, because a microorganism will grow until an environmental or nutrient factor limits its growth. In this case, a nutritional factor limited the growth of the microorganisms due to the depletion of carbon source within the media. When the substrate concentration was increased to 0.73%, it was observed that the OD readings continued to increase until the seventh day, then became constant. This implied that by increasing the concentration of the substrates, the isolates were able to live longer within the media. On the other hand, when the substrate concentration was doubles to 1.5%, there was no growth. This may be due to the fact that the condition became toxic or the bacteria was unable to cope with the higher organic load. These results were further accompanied by COD readings as indicated in Fig. 2.

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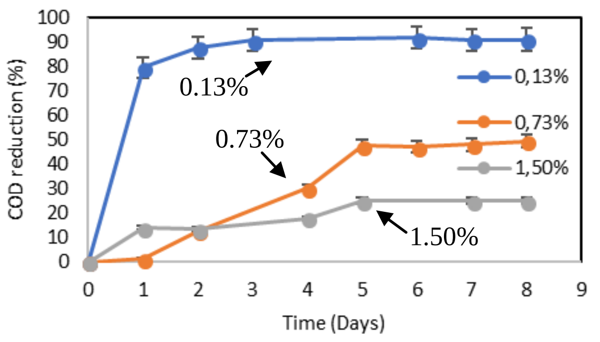

Fig. 2: Effect of substrate concentration on COD reduction

After 3 days of incubation, 90.7% of the COD was reduced in the flask that contained 0.13% carbon source. This was followed by 49.4% and 24.98% COD reduction at substrate concentration of 0.73 and 1.5% respectively. Although the bacteria survived longer in the solution that contained 0.73% CS, the bacteria were not degrading the compounds as much as the ones inoculated in 0.13% carbon source. Although no growth was seen in Fig. 1, however there was 24,98% COD removal indicating that bacteria were present and degrading very slowly.

B. *Effect of temperature*

A study on the effect of temperature on COD reduction was done using 3 incubators at 3 different temperatures (15, 25 and 35°C).

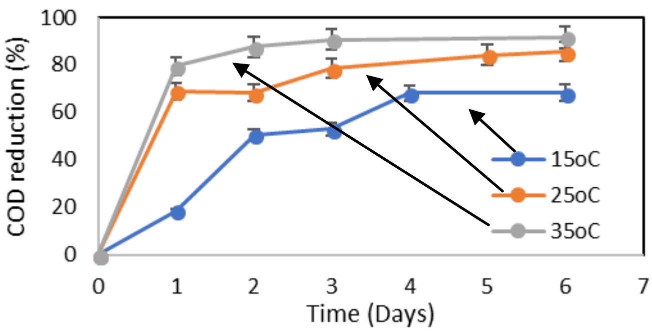

Figure 3: Effect of temperature on COD reduction

It was observed that at the highest temperature (35°C), highest COD removal (91.7%) was achieved. At lower temperatures of 25 and 15°C, the highest achievable COD removal was 85.6 and 68% respectively. This is an indication that the biodegradation of SCA and VFA's was done by mesophiles. To confirm the effect of temperature on bacterial growth, four plates were prepared with nutrient agar and inoculated with bacteria, using an inoculation loop. The plates were incubated for 24 hours and are shown in Fig. 4. The control plate, with no inoculum, had no growth after 24 hours (Fig. 4 a), indicating a sterile condition. Plates inoculated with bacteria showed a direct increase in growth with an increase in temperature. The growth of inoculum was identified by the smear on the plates as circled in red on Fig. 4 b and c. The entire plate which was incubated at 35°C had inoculum (Fig. 4 d). This confirmed the results obtained from Fig. 3., and the conclusion that the bacterial species involved in this study is mesophilic. It was reported that cell growth is affected by temperature [10]. At elevated temperatures, cells tend to function better than at low temperatures. This is because a cell membrane is formed by two layers of fatty acid membranes called phospholipids. Phospholipids within a membrane help to keep it fluid and semi-permeable so that all the necessary nutrients can enter the cell membrane, whilst harmful substances are kept out [10].

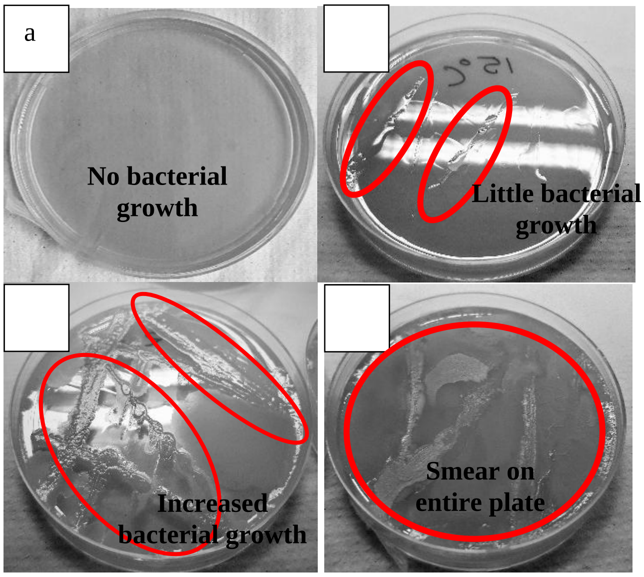

Figure 4: Agar plates of bacteria grown at different temperatures. a) Control, b) 15°C, c) 25°C, d) 35°C.

Elevated temperatures increase the fluidity of the cell membrane and thus more nutrient can penetrate the cell membrane and increase the growth rate of the cell. The opposite is then experienced at lower temperatures [10].

IV. BACTERIA IDENTIFICATION

According to biochemical characteristics and 16S rRNA sequence analysis the isolates were identified as *Lactobacillus murinus*, *Bacillus amyloliquefaciens*, *Bacillus velezensis*, *Lysinibacillus* sp., *Bacillus pumilus*, *Enterobacter xiangfangensis*, *Enterobacter hormaechei* subsp. and *Enterobacter cloacae* strain. The bacillus strain was the dominant species in the 16S rRNA identification. It has been reported that this strain is capable of growing in different types of wastewaters, regardless the conditions of the wastewater [11]. *Enterobacter* spp. are bacterial strains closely related to the family of *Enterobacteriaceae*. They are gram negative rod-shaped strains with the *Enterobacter cloacae* complex [12].There are 23 strains of this bacteria of which 22 were isolated from humans [13]. The presence of this bacteria may arise from the fact that upstream of the wetland sewage is discharged into the wetland.

V. RECOMMENDATIONS

More studies need to be done to tackle all the compounds found in FT wastewater i.e. hydrocarbons and long chain alcohols. The bacteria consortium has been shown to

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degrade the SCA and VFAs present in FT, however at higher concentrations they need to slowly acclimatize to these compounds.

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