

# Studies on Biofiltration of Contaminant Mixtures

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## ABSTRACT

Most gaseous emissions from waste water treatment plants, industrial operations, and solids handling facilities, consist of contaminant mixtures rather than pure chemicals. In the literature, several studies have been conducted on biological treatment of gaseous emissions using either naturally bioactive media (soil, peat, compost, etc.) in biofilters or synthetic support media in biotrickling filters. Generally, these studies have relied on single contaminants. However, previous studies, conducted by the author, on biodegradation of mixtures in liquid phase, has shown that biodegradation rates for mixtures are quite different than single contaminants, mainly due to either synergistic or antagonist relationships between the main bacterial species responsible for the degradation of the various compounds.

In this paper, a systematic study was initiated to understand biodegradation of gaseous contaminant mixtures in biotrickling filters and develop theoretical models that would provide avenues for extending pure compound studies to compound mixtures. This understanding is very important since there are hundreds of gaseous contaminants which can constitute million sof gaseous contaminant mixtures, and it is impossible to experimentally determine the biotreatment kinetics of every possible mixture combination. Results of the theoretical and experimental studies have been presented in this paper.

## INTRODUCTION

There are four types of responses that microorganisms have in the presence of multiple substrates: diauxic two-phase with an diauxic lag phase; diauxic two-phase without a lag phase; simultaneous utilization of more than one substrates; and utilization of only one substrate. The classic response is that of the diauxic two-phase utilization of substrates with a diauxic lag phase between the two utilization phases. The microorganisms are assumed to consume the favorite substrate first until its depletion. After the first phase, enzymes for the second substrate are produced and accumulated to a certain level that corresponds to the diauxic lag phase. With the necessary enzyme level, the microorganisms present the second phase of metabolism that utilizes the second substrate as its sole resources. The typical example of the second phase of metabolism is the batch growth of *E. coli* on glucose and lactose that was first demonstrated by Monod<sup>1</sup>. *E. coli* grows on glucose preferentially in the presence of two sugars. After the depletion of glucose, there exists a lag phase followed by the second phase of utilization of lactose. The process of batch growth is fairly common, as witnessed by the batch growth of *E. coli* on glucose and xylose<sup>2</sup>.

However, not all diauxic two-phase preferential utilization of two substrates exhibit a diauxic lag phase. It is believed that either the microorganisms use one enzyme to catabolize two similar substrates or the enzyme for the second substrate is present in the first phase of utilization. The batch growth *E. coli* on the mixtures of glucose and galactose<sup>2</sup> as well as glucose and fructose<sup>3</sup> belong to this category. The sequential utilization of a substrate does not show up in the diauxic lag phase.

Microorganisms have the potential to grow on two different substrates simultaneously. There are two subclasses in this category. One is when the two substrates serve as two different functions in microorganism metabolism. These two substrates are termed as complementary substrates. One example is the growth of microorganism *E. coli* on glucose and ammonium<sup>4</sup>. Glucose serves as carbon source and ammonium serves as electron donor. Another subclass of simultaneous utilization of substrates is when two substrates serve the same function in microorganism growth. In such a case, these two substrates are termed as substitutive substrates. One example of the substitutive substrate is the growth of *P. vulgaris* on glucose and sodium citrate<sup>5</sup>, where both glucose and citrate serve as carbon and energy sources. The two substrates are utilized simultaneously. When the two substrates differ significantly, the microorganisms may use only one substrate. It may take too long of a time for the microorganisms to adapt to the second substrate, consequently, the microorganisms seem not utilizing the second substrate at all in the time period of investigation.

Building on the above-mentioned research, experiments are designed, in both single and multiple compounds biofiltration, using mixtures of methanol, ethanol, acetone, acetaldehyde, propanol, iso-propanol, and butanol. These compounds are selected because they are common environmental pollutants, they include chemicals that have variety of biodegradability, and most of them do not require a long period of time to acclimate the biomass. The biomass is acclimated, in continuous biofilter, to attain to a stable capability to consume the substrates before it is used for dynamic biofiltration tests. These tests are carried out in microbiofilter systems, which are basically a batch-type reactor system. The batch type system was chosen because of two reasons: 1) the performance of a continuous biofilter would be affected by other processes such as sorption and adsorption of contaminant chemicals as well as flow patterns of liquid/nutrient flow; and 2) the biofilter height would be very high in order to study less biodegradable chemicals. In a batch system, effects of those processes other than biodegradation are well controlled and only the operation time changes for chemicals of different biodegradabilities. Experiments are designed in order to obtain meaningful results that can be employed to answer basic questions regarding biofiltration of mixed contaminants in biofilters.

## **EXPERIMENTAL STUDIES**

The study of mixed-substrate biodegradation in biofilters is conducted using a microbiofilter systems, shown in Figure 1. The system consists of a reservoir tank, a reactor column, and a circulation pump. A microbiofilter is a differential bioactive reactor column. The packing of this column is usually taken from a biofilter that operates in

continuous steady state conditions. The system gas is stored in a glass reservoir tank. Substrates are injected into the tank and the gas can be sampled from a sampling port. The circulation pump keeps the small amount of media in the microbiofilter to be exposed to a gas phase with almost the same concentration as that of the gas storage tank.

**Figure 1 A schematic of a microbiofilter system.**

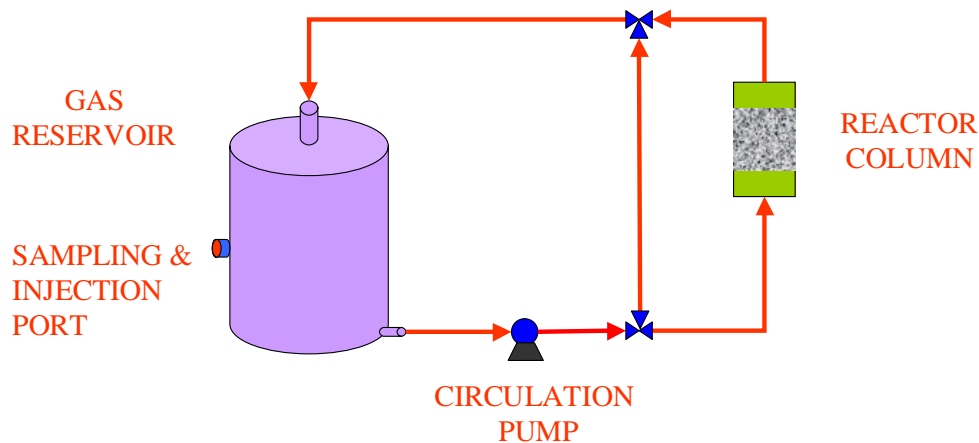
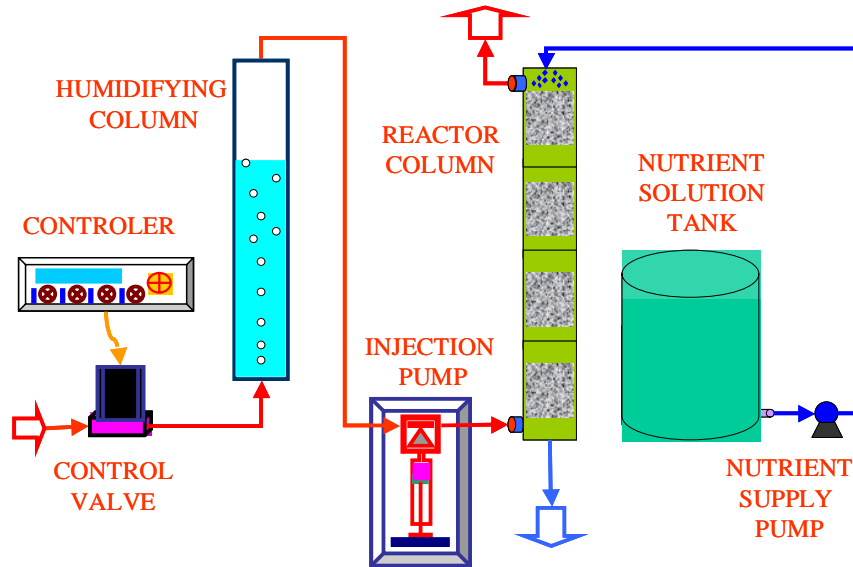


Figure 2 shows a schematic of the steady state biofilter that acclimates and grows biomass for microbiofilter tests. A biofilter system consists of a humidifying column, a syringe injection pump, a reactor column, a nutrient solution tank and a solution supply pump. The reactor column could be a biofilter or a trickling biofilter. Microorganisms from activated sludge or compost may be used to seed the columns. These bacteria can attach to the media surface in the form of biofilms and grow on the substrates under conditions of sufficient nutrients and proper pH level. The nutrient solution is usually conditioned in the nutrient tank and circulated by the circulation pump and distributed to the column through a liquid nozzle spray at the top of the column.

Carbon dioxide evolution is taken as a measurement of biomass activity and substrate consumption. Although CO<sub>2</sub> is not directly represent the biomass activity, there is no better way to relate the substrate biodegradation in a biofilter. Usually, biomass weight is measured to assess the biomass growth, but this kind of measurement can neither be accurate nor be directly correlated to biomass growth.

Gas are sampled and analyzed in a gas chromatography. Substrate concentrations are analyzed with a HP GC with FID detector. The HP GC is equipped with an ALLTECH s.s. CHROM WAW 80/100 mesh column. In this system, nitrogen serves as the carrier gas. Carbon dioxide is analyzed through another HP GC equipped with a TCD detector. Helium serves as carrier gas with a flow rate set at 25 ml/min. The sample gas volume measures at 0.4 ml. These GCs are calibrated before they are used to analyze samples from each test.

**Figure 2 A schematic of a steady state biofilter that grows and acclimates the biomass for microbiofilter tests.**



The differential reactor column is taken from a biofilter where the biomass is acclimated in a continuous steady-state condition. The biofilter was stopped feeding 12 hours before a microbiofilter test in order to make sure that the growth limiting substrate is completely degraded. The microbiofilter system is humidified before each test. Investigated substrate is injected into the system before the differential reactor is placed. The concentrations of the substrate and CO<sub>2</sub> evolved from biomass metabolism are measured during the test. The history of substrate consumption and the rate of CO<sub>2</sub> evolution can be used to calculate cell growth utilization of the substrate. Multiple substrate utilization in a microbiofilter system will show sequential or simultaneous decreases in their concentration inside the system.

With all selected compounds, the following operations and tests are carried out in order to study the performances of biomass utilization of mixed substrates.

- 1) Single substrate test with biomass acclimated with ethanol
- 2) Dual substrate test with biomass acclimated with ethanol
- 3) Three substrate test with biomass acclimated with ethanol
- 4) Endogenous respiration study of biomass in biofilm at the absence of substrates
- 5) Online single substrate injection and response test after endogenous respiration phase
- 6) Online multiple substrate injection and response test of less favorite substrate after favorite substrate

- 7) Online multiple substrate injection and response test of favorat substrate after less favorate substrate utilization.

Single substrate tests are carried out in order to get the kinetic parameters of biomass utilization of each substrate. Dual substrate tests are used to see if the biomass uses pair of substrates sequentially or simultaneously. Three substrate tests are carried out in order to see any triauxic biomass utilization of substrates. Endogenous respiration experiments are designed to see the extent of endogenous respiration and to answer if utilization of substrates stops endogenous respiration.

Endogenous respiration tests measure the CO<sub>2</sub> evolution rate at the absence of any substrate in the system. Endogenous respiration phase followed by single substrate injection and response test is implemented first with endogenous respiration test. While the biomass is at a constant CO<sub>2</sub> evolution rate, a substrate is injected into the system. The substrate concentration and CO<sub>2</sub> concentration are measured. The comparison of CO<sub>2</sub> evolution rates at the absence and in the presence of a substrate may answer the question we asked above.

Online injection and response tests are designed to see if the biomass would stop using less favorite substrate and start using favorite substrate and if the biomass would use less favorite substrate when it is injected or whether the biomass is using more favorite substrate. Online multiple substrate injection and response tests are carried similar to online single substrate injection and response tests, except that more than one substrates are injected, either at the same time or at different time, into the system. The substrate concentrations and CO<sub>2</sub> concentration are sampled and measured during the dynamic process.

## **RESULTS AND DISCUSSION**

Data collected includes results from batch process tests, injection and response tests in microbiofilter, for both single and multiple substrates biofiltration. Some typical and illustrative data are presented in Figure 3 through Figure 14 and conclusive findings are summarized here to answer the basic questions raised earlier in this paper. From the biofiltration of methanol, ethanol, acetone, acetaldehyde, propanol, iso-propanol, and butanol in microbiofilters, the following results are obtained.

- 1) Multiple substrates can be consumed simultaneously in microbiofilter biofiltration;
- 2) Biomass utilizes these compounds at different rates and has an order of: acetaldehyde > ethanol > propanol > iso-propanol, acetone, methanol > butanol
- 3) The contribution to the total biomass maintenance and growth, represented by the production of CO<sub>2</sub>, by each compound can be additive, but the total effects on biomass behavior is likely to be limited by a maximum bacterial growth rate.

Simultaneous transformation of multiple substrates in microbiofilter biofiltrations is demonstrated, in both batch tests, injection and response tests, by the biofiltration of ethanol and acetone as in Figure 5, 8, 11, ethanol and propanol as in Figure 11 methanol, ethanol and acetone as in Figure 14, and ethanol and acetaldehyde as in Figure 5 and 7. These dynamic performances of mixed substrate biofiltration exhibit that multiple substrates are observed to be simultaneously transformed and removed in the process of biofiltration in microbiofilters. The biofiltration of methanol and ethanol in Figure 13 shows possible inhibition of methanol biodegradation by ethanol.

The differences of substrate transformation rates can also be observed from multiple substrate biofiltration dynamics. Figure 4 shows that butanol is not biodegraded with a four-day test. Propanol is removed in the system very quickly, as in Figure 11. However, the CO<sub>2</sub> production rate during that time almost does not change promptly. The effect of propanol biodegradation on CO<sub>2</sub> production exhibits gradually as is evidenced by the CO<sub>2</sub> concentration change in the system. Therefore, it is likely that propanol is first quickly transformed into an intermediate product and that product is then biodegraded slowly and finally ends up in CO<sub>2</sub>, water, and biomass.

Figure 6 shows that iso-propanol is obviously quickly transformed to acetone, which is then biodegraded by the biomass while some acetone being released to the bulk gas phase. Acetone in the system is identified as well as analyzed during the tests. The figure shows that iso-propanol concentration decreases while acetone concentration increases. After iso-propanol is completely converted, the acetone concentration in the system starts to decrease because of its biodegradation that results in an increase in CO<sub>2</sub> concentration in the system.

Acetone degrades in microbiofilters slowly, as is demonstrated in Figure 4, 8, 9, and 12. Its consumption gets faster in later tests of microbiofilter biofiltration, as indicated by the comparison of Figure 14 and previous figures. These results show that acetone is biodegraded more slowly than ethanol and both are consumed simultaneously in the microbiofilters.

Acetaldehyde exhibits a faster consumption rate than ethanol, as shown in Figure 5 and 7. It seems that among the compounds tested, acetaldehyde is the only one whose consumption rate is higher than ethanol.

The experiments show that methanol can be biodegraded at a rate close the biodegradation rate of acetone. Its consumption is relatively slow, but like acetone, its utilization seems directly result in CO<sub>2</sub> production. Figure 13 and 14 show some dynamic of methanol biofiltration in microbiofilters.

Combined contribution to biomass maintenance and growth by multiple substrates can be observed from experimental results from both batch tests as well as injection and response tests. Figure 4 shows that acetone and ethanol concentration decrease continuously in a biofiltration process of a mixture of ethanol and acetone in a microbiofilter. When both substrates are present, CO<sub>2</sub> production rate is high. The rate

decreases to a lower level after ethanol is completely removed. Figure 12 shows the dynamics of an injection and response test of acetone and ethanol. Increases in CO<sub>2</sub> production rate can be seen in both times when ethanol is injected. Also observable decrease in CO<sub>2</sub> production occurs when ethanol is finished in the system. Since both substrates are transformed simultaneously, it is very likely that they also contribute to the CO<sub>2</sub> production of the system. Figure 14 shows an injection and response test of methanol, ethanol, and acetone. Methanol and acetone can be seen removed simultaneously in the microbiofilter. The CO<sub>2</sub> production rate increases right after the mixture is injected into the system that has been in a state of endogenous respiration condition. The rate decreases when acetone is first depleted. It decreases further when methanol is depleted. There is no doubt that methanol is being consumed at the presence of acetone and therefore, both substrates must have been utilized for bacterial maintenance and growth. This microbiofilter biofiltration dynamics shows a combined contribution of both acetone and methanol to the production of CO<sub>2</sub>, therefore to the maintenance and growth of the biomass.

While the experimental results show that multiple substrates can contribute together to the growth and maintenance of biomass, experiments also demonstrate that the total CO<sub>2</sub> production rate in a multiple substrate biofiltration can be limited by a maximum growth rate. This phenomenon can be observed in a batch biofiltration test of a mixture of ethanol and acetaldehyde, as shown in Figure 5. When acetaldehyde is depleted, the CO<sub>2</sub> production does not exhibit any slower trend than that when both acetaldehyde and ethanol are present in the system. This kind of phenomenon can also be seen in an injection and response test of ethanol and acetaldehyde, the dynamics is shown in Figure 7. When acetaldehyde is injected into the system that already has ethanol in it, the system does not exhibit an observable increase in CO<sub>2</sub> production rate. However, in single substrate injection and response tests of ethanol and acetaldehyde, significant increases in CO<sub>2</sub> production rate are observed when the substrate is injected.

The possible limitation of a maximum growth rate or a maximum CO<sub>2</sub> production rate can also be seen in the injection and response tests of acetone and ethanol. Figure 8 shows a dynamics of an injection and response test of ethanol and acetone. The CO<sub>2</sub> production does not increase when acetone is injected into the system where ethanol is already present. On the other hand, as shown in Figure 9, CO<sub>2</sub> rate increases to a much higher level when ethanol is injected into a system that has acetone. Therefore, a limit on the CO<sub>2</sub> production exists very likely and when multiple substrates in a system can contribute to the growth of biomass more than that limit, their real contributions are regulated to the maximum growth rate or CO<sub>2</sub> production rate limitation.

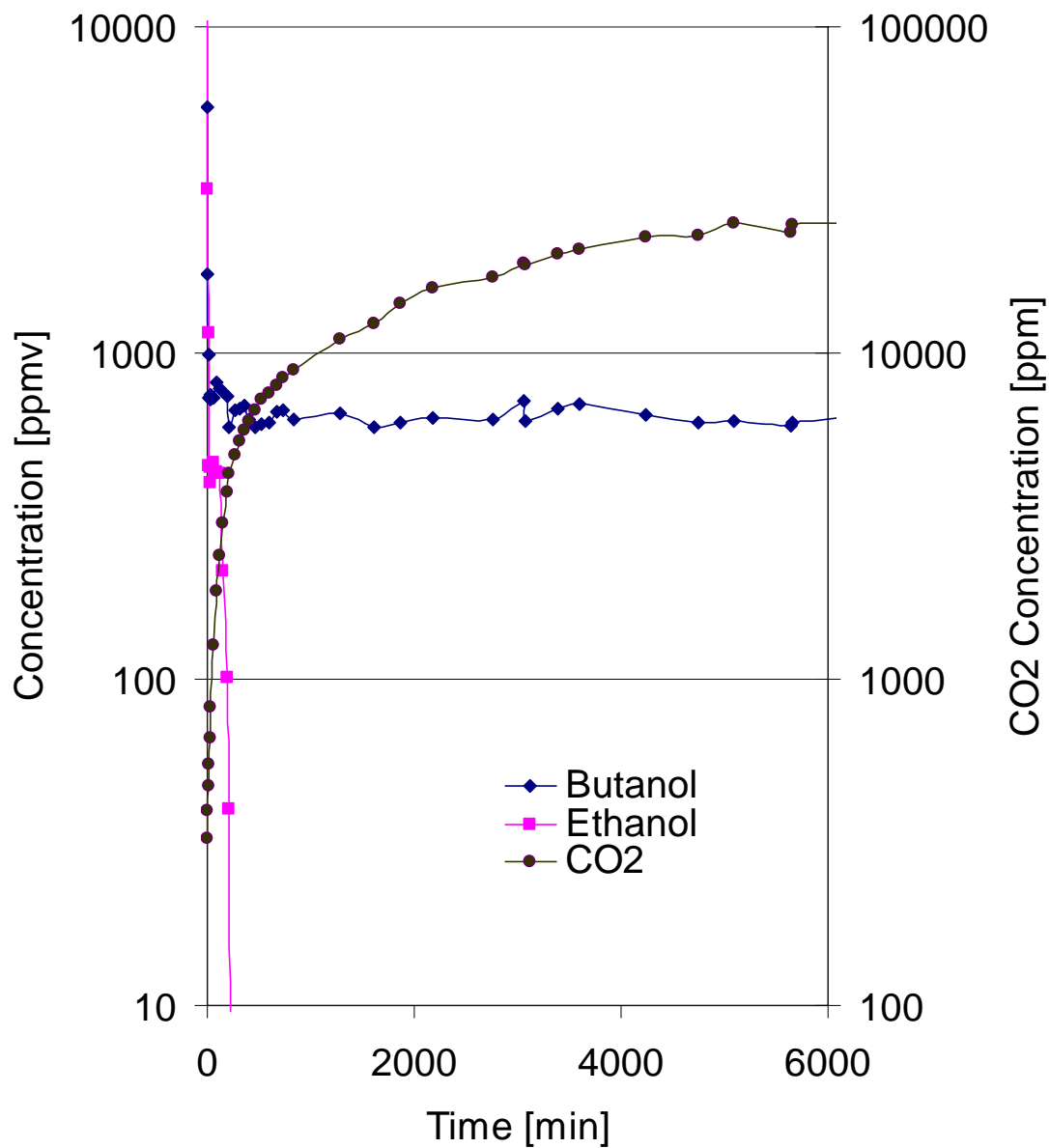
## CONCLUSIONS

Based on studies of bacterial growth on multiple substrates in suspended cultures, experiments of mixed contaminants biofiltration in biofilters are designed and these experiments are conducted in microbiofilter systems. This study shows that, in studying biofiltration of multiple contaminants, microbiofilter systems have advantages over a continuous biofilter and they can be used as a tool for investigating bacterial behavior in a changing environment of waste gases. The injection and response tests also proved to be an effective method that can be used to quickly change the conditions of a microbiofilter system and to observe the dynamic response of the biomass to the activating environment. The biofiltration of selected compounds indicate that the biomass utilizes the contaminants present in the system simultaneously although it has different preference to and uses different utilization rates for different substrates. The utilization rates of these substrates by the biomass are: acetaldehyde > ethanol > propanol > iso-propanol, acetone, methanol > butanol. The experiments show that the biomass does not utilize butanol in four days, iso-propanol is first transformed to acetone by the biomass and then acetone is utilized in the biofilter system, as well as that acetone and methanol are utilized in approximately the same rates. Experimental results indicate that it is likely that biomass maintenance and growth are contributed by all the substrates present in the system, but the total effects is limited by a possible maximum rate of bacterial growth. More detailed understanding of the behaviors of bacteria in a biofilm in the presence of multiple contaminants may be obtained by mathematical modeling and simulation of the experiments conducted in this research and presented in this paper.

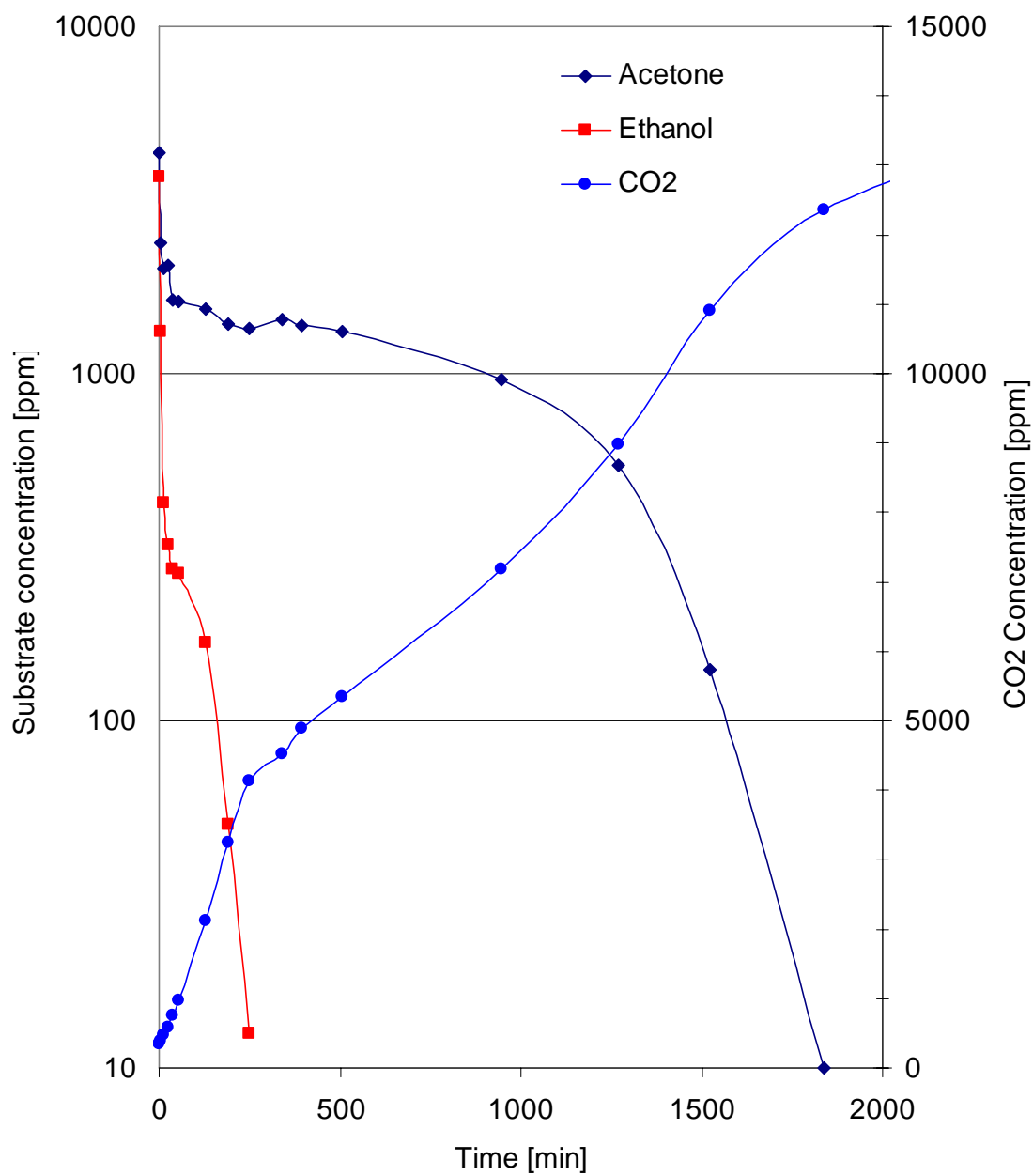
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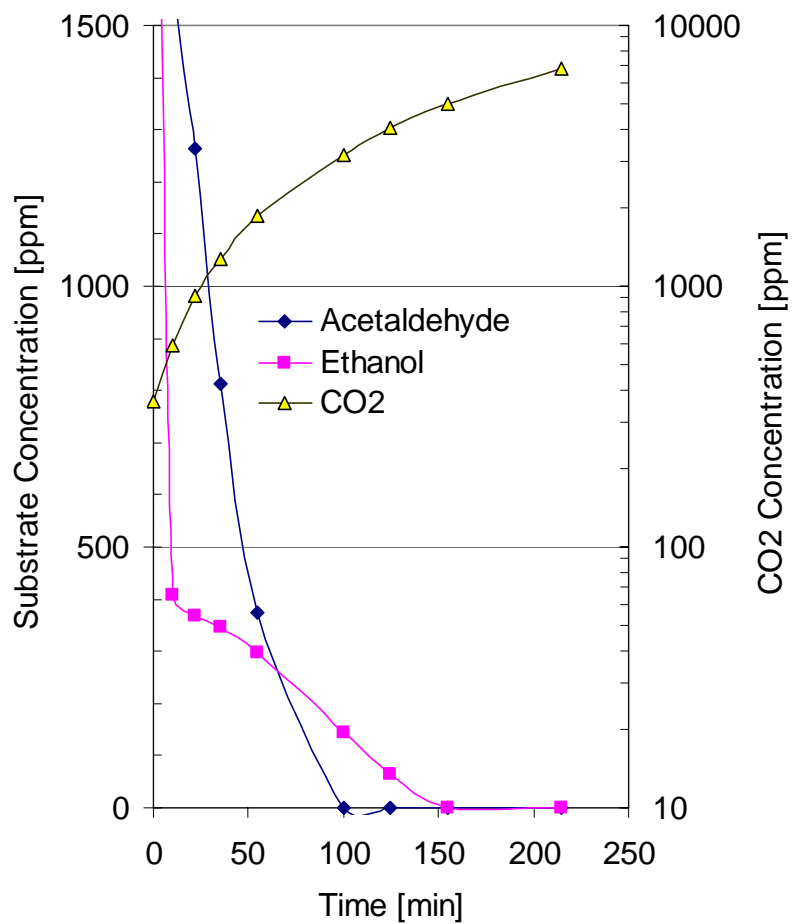
**Figure 3. The Biofiltration dynamics of a mixture of ethanol and butanol in a microbiofilter system. Butanol has not been biodegraded in four days.**



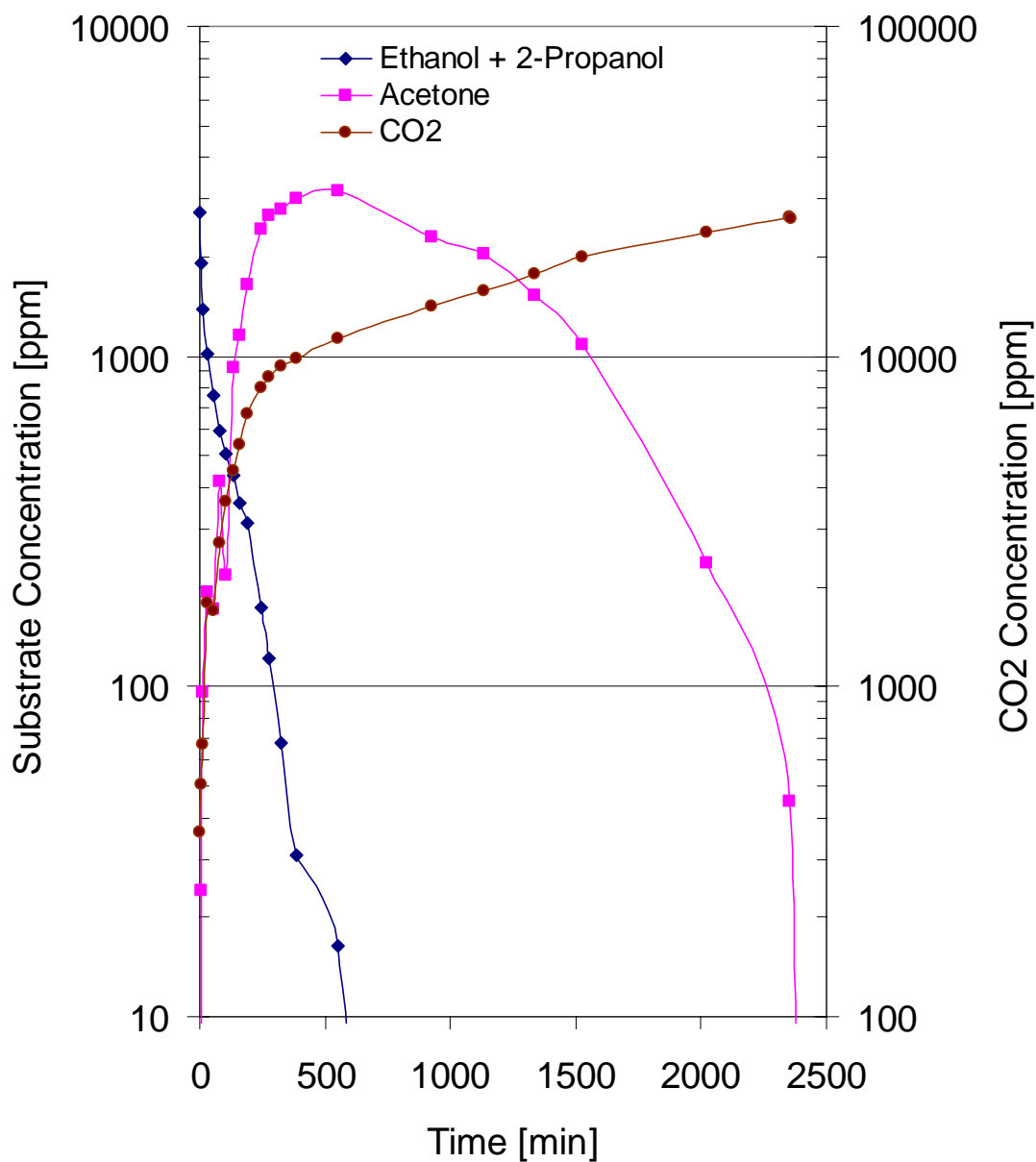
**Figure 4.** The biofiltration dynamics of a mixture of ethanol and acetone in a microbiofilter.



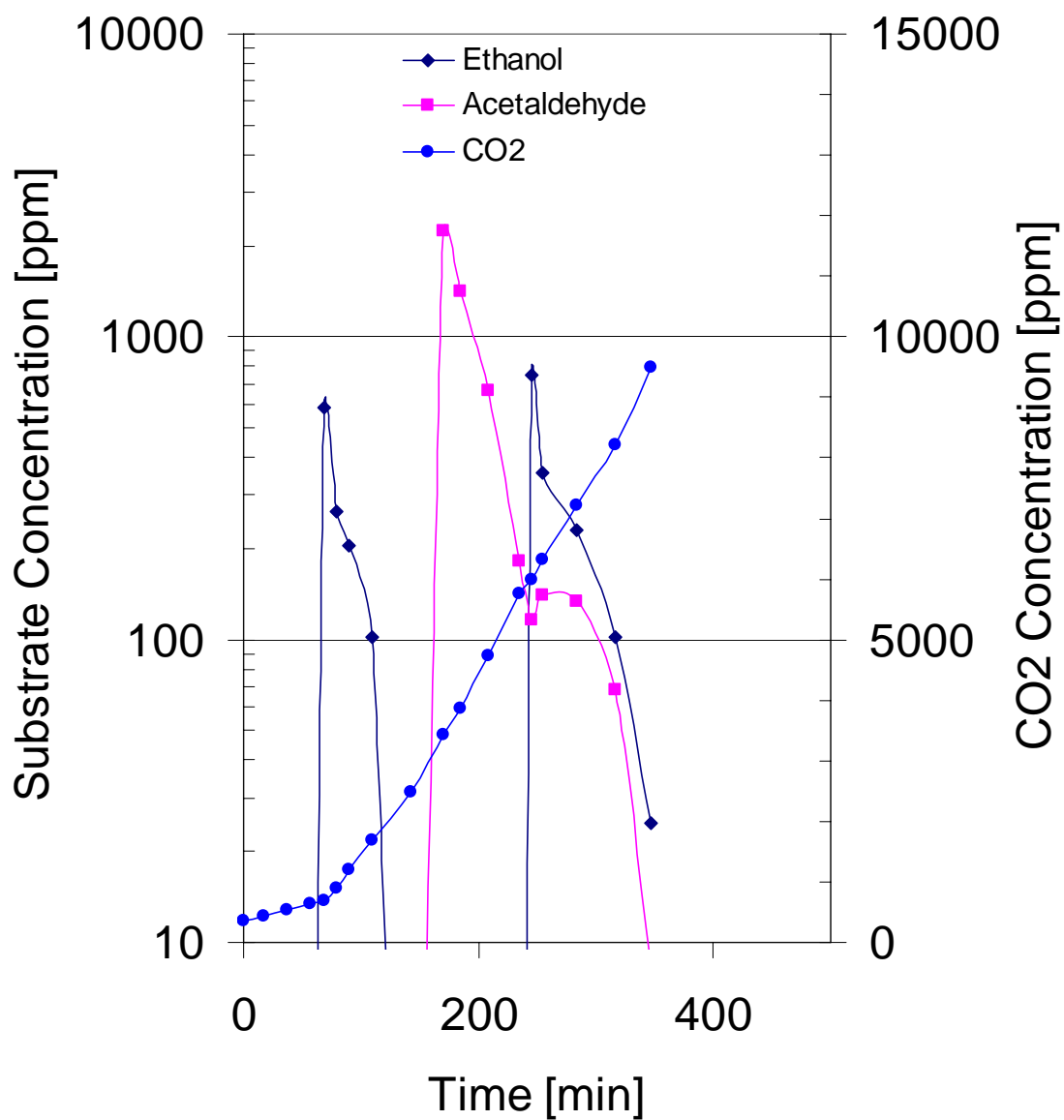
**Figure 5. Concentration changes of acetaldehyde and ethanol in multiple substrate biofiltration in a microbiofilter.(CO<sub>2</sub> not in log scale)**



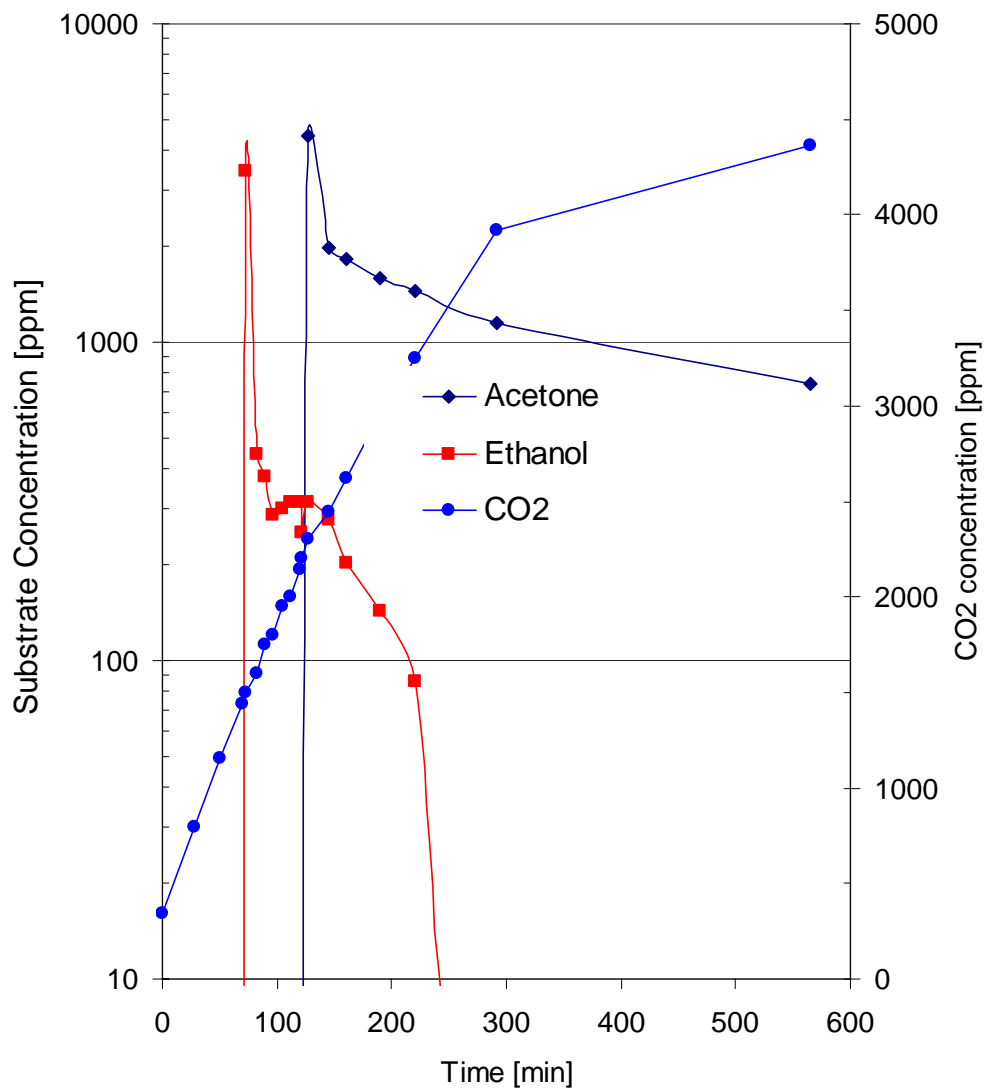
**Figure 6.** The biofiltration dynamics of a mixture of ethanol and 2-propanol in a microbiofilter. Acetone is first produced as an intermediate product of 2-propanol conversion followed by a period of biofiltration of acetone.



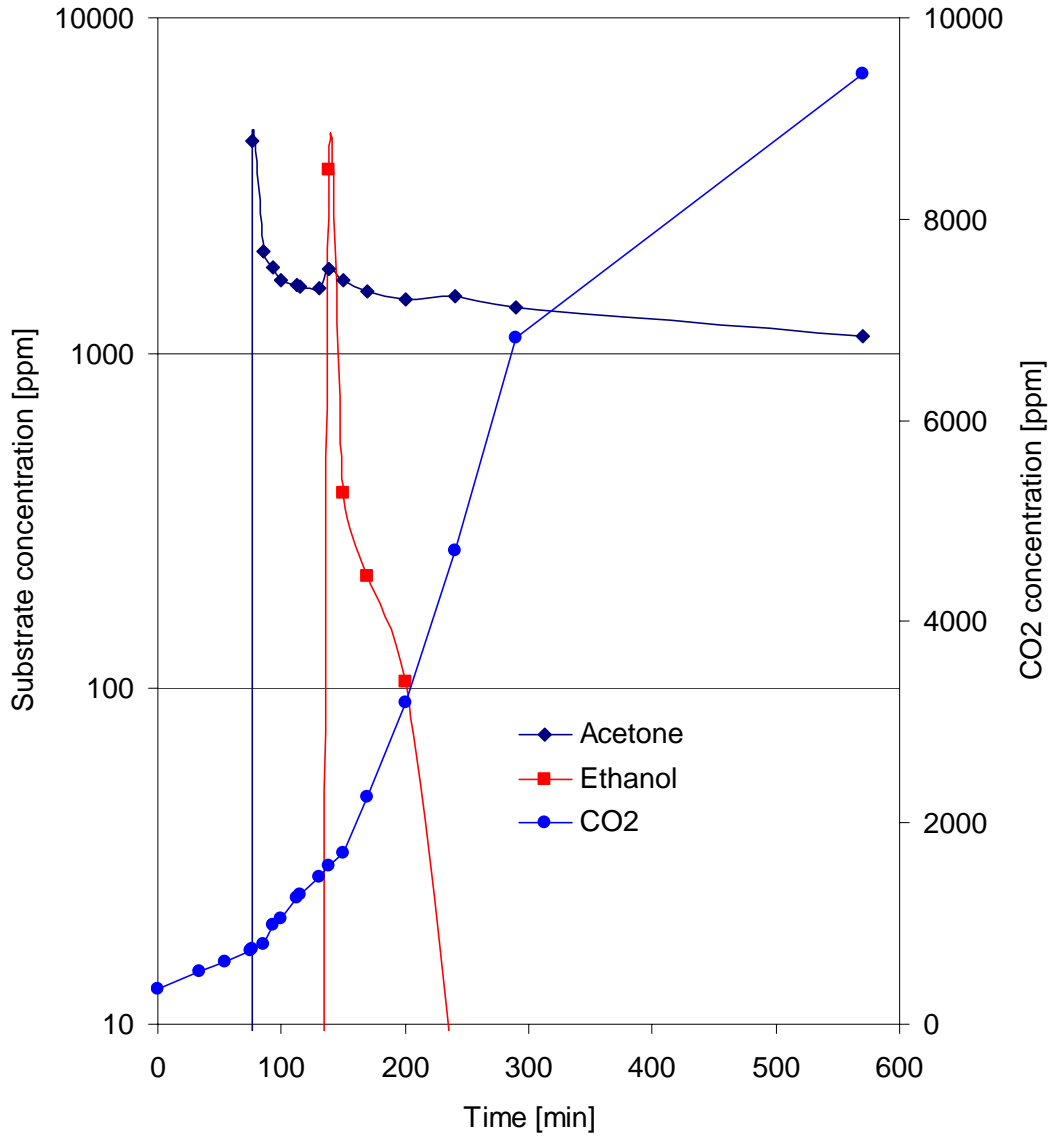
**Figure 7. An injection and response test of ethanol and acetaldehyde in a microbiofilter. Ethanol is injected after a short time of endogenous respiration period. Acetaldehyde is injected into the system after ethanol has been completely biodegraded. While acetaldehyde is being degraded, ethanol is introduced into the system again.**



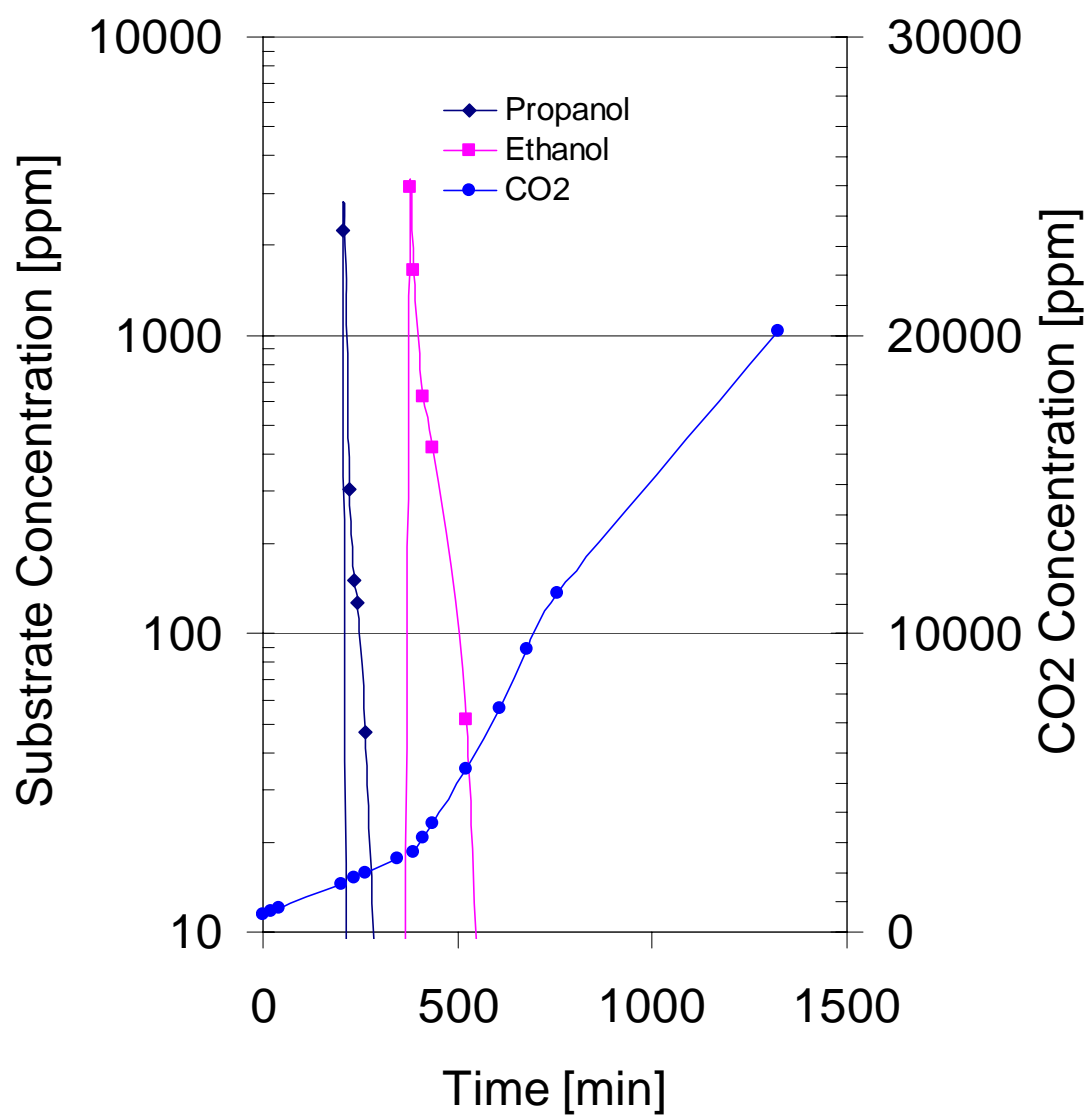
**Figure 8. An injection and response test of ethanol and acetone. Ethanol is injected into the system after a period of endogenous respiration process, and then following the introduction of the second substrate acetone.**



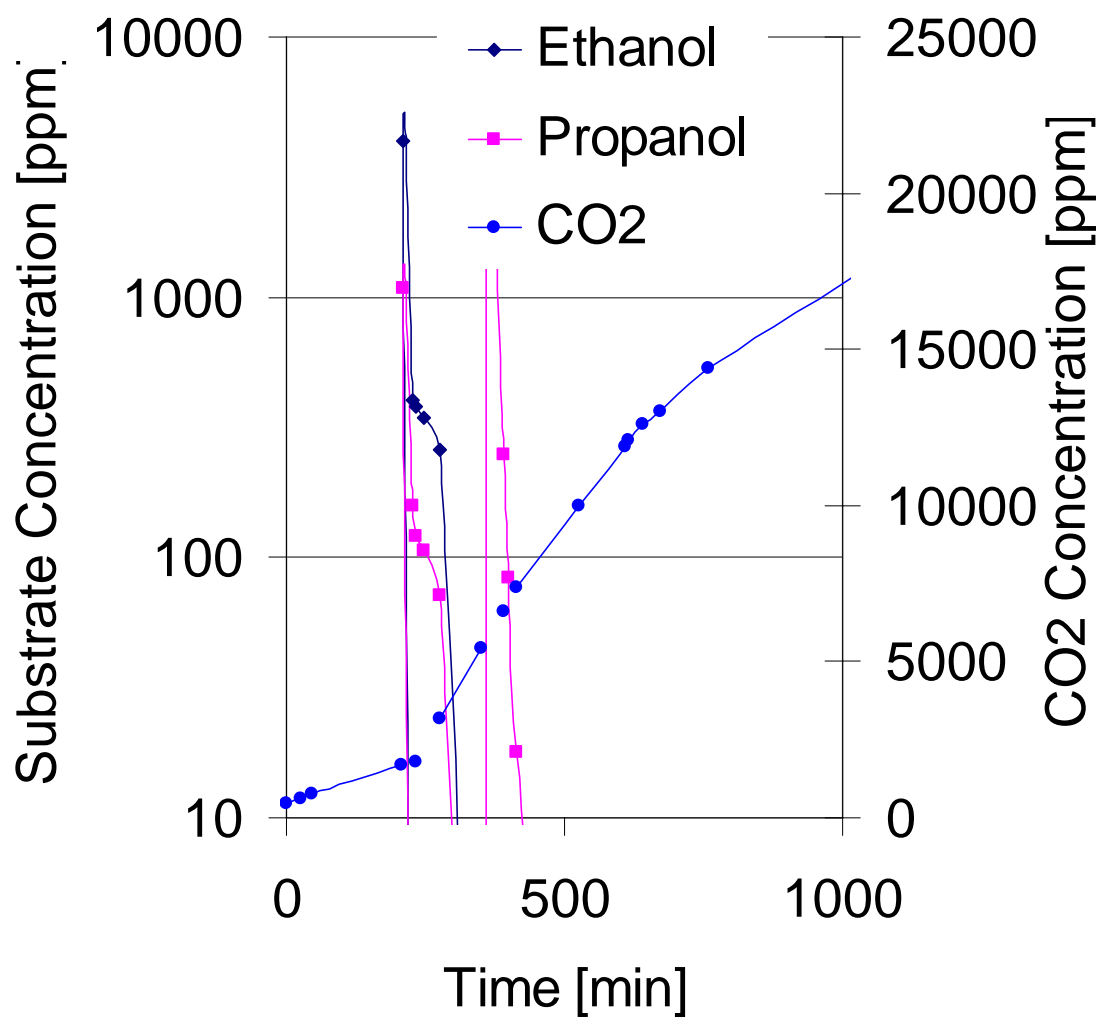
**Figure 9. An injection and response test of ethanol and acetone. Acetone is injected first after a period of endogenous respiration and then following the introduction of ethanol.**



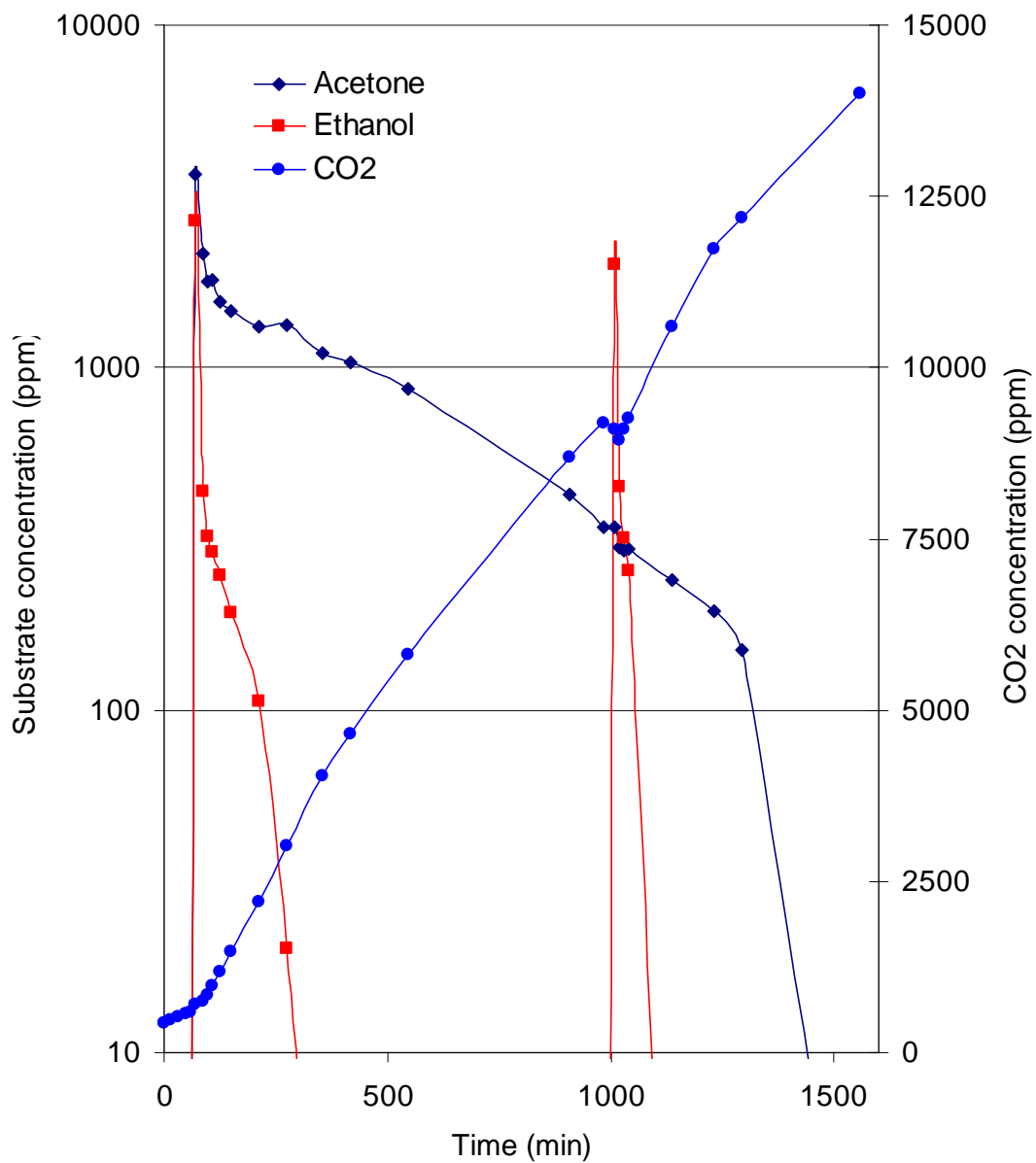
**Figure 10. An injection and response test of propanol and ethanol in a microbiofilter. Propanol is the first chemical injected into the system after it experiences a period of endogenous respiration process.**



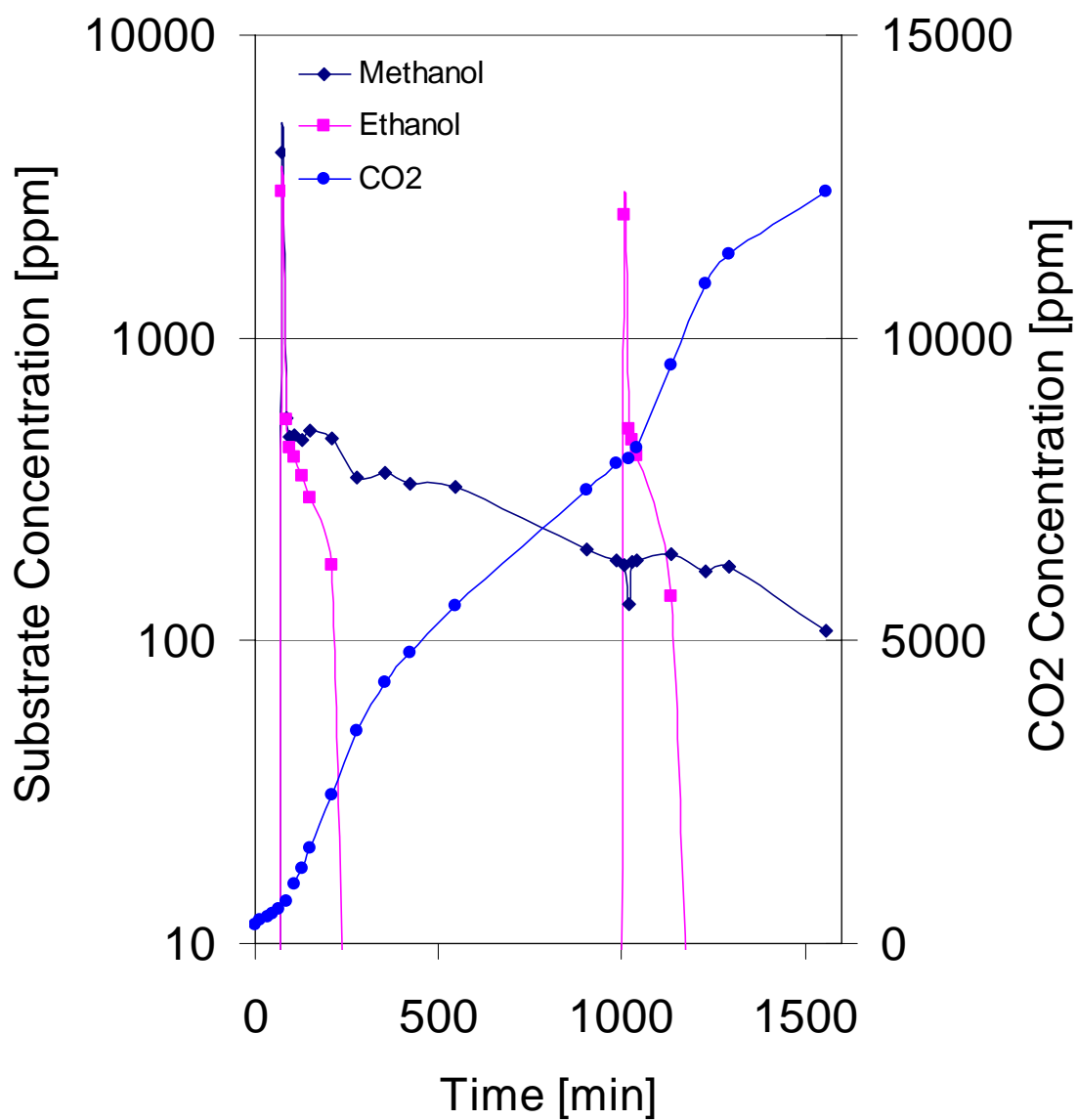
**Figure 11. An injection and response test of ethanol and propanol. Both substrates are biodegraded simultaneously at approximately the same rate.**



**Figure 12. An injection and response test of acetone and ethanol. Simultaneous consumption of both substrates is observable.**



**Figure 13. An injection and response test of methanol and ethanol. A mixture of methanol and ethanol is injected into the system after the system has operated under endogenous respiration condition. A second dose of ethanol is injected 12 hours after the first portion is completely depleted.**



**Figure 14. An injection and response test of methanol, ethanol, and acetone. A mixture of methanol and acetone is first injected into the system that is originally in a process of endogenous respiration. Ethanol is introduced when both methanol and acetone are completely finished in the system.**

