A Chemostat competition experiment

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Abstract

Two chemostats were run at slightly different pump speeds to follow the competition of two semi-pure cultures both fermenting glucose. One of the cultures, an amino acid fermenter (AAF) was isolated from a casaminoacid-rich plate and the second culture, black gold (BG), originated from sludge from a zinc mine waste water treatment reactor. BG has a lower growth rate than AAF, so AAF was expected to win the competition for the substrate. One chemostat did not reach steady state, due to the slow flowrate. The other chemostat worked well, reached steady state and kept a low substrate concentration. However, there was no change in population observed after 3 days. Most likely it takes longer to outcompete BG and get a dominating population of AAF.

Introduction

A chemostat is an open system with continuous growth of microorganisms. Fresh medium is introduced at constant flow speed (f) and culture liquid is removed at the same rate according to hydrolic principles. The dilution rate (D) of the chemostat is defined as the flow speed per volume (f/V). A steady state system is reached when the applied dilution rate equals the bacterial growth rate (μ). Then the rate of change in bacterial concentration equals the rate of increase in bacterial concentration (μx) minus the washout rate (Dx). Growth of cells in a chemostat is limited by the rate at which a particular limiting nutrient is supplied. When a mixed population of microorganisms is inoculated into the chemostat, selection is determined by the way in which growth rates (μ) depend on concentration of substrate (s). That in turn depends on the applied dilution rate. The organism with the highest growth rate at the applied dilution rate becomes dominant. Slow growing organisms are washed out.

The goal of this research was to learn how to build and run a chemostat, and to carry out and follow a competition experiment between 2 organisms competing for the same substrate and producing different fermentation products. For this experiment a chemostat was inoculated with 2 semi-pure cultures, AAF and BG, to follow the competition. The semi-pure cultures are obtained from enrichments in a glucose medium by a dilution to extinction technique. The AAF enrichment, an amino acid fermenter, is coming from a colony on a casaminoacid rich plate. The BG (black gold) enrichment is inoculated with material from an expanded granular sludge bed reactor (EGSB) in Kennecott, near Salt Lake City. This reactor is processing waste water from a zinc mine (black gold). The water contains zinc, copper, iron, and other

heavy metals, metal sulfides and –carbonates. The reactor is fed with H_2/CO_2 and ethanol and run at 30-35 degrees Celcius at pH 7.5-8. If there are glucose fermenters in this reactor, they will acidify the reactor liquid by the acidic products they make. The low pH together with the produced H_2 will inhibit the methanogens from doing their job later on in the process. So it is interesting to see if there are any glucose fermenters in this sludge and what products they make when they degrade glucose.

Growth rates were determined in batch culture and product analysis were performed on HPLC prior to the inoculation of the chemostat. The chemostat was run for 4 days at constant flow speed and sampled for microscopic determination and product analysis. The dilution rate of the chemostat was calculated to be similar to the average growth rate.

Materials and Methods

Two separate enrichments, named amino acid fermenter (AAF) and black gold (BG), were made in a medium containing basic salts (NH_4Cl, NaCl, $MgCl_2$, KCl, and $CaCl_2$), trace elements, phosphate buffer (NaH2PO4/Na2HPO4), yeast extract, and resazurin as a oxygen indicator. Dextrose (glucose) served as a substrate and was added to give a final concentration of 20mM in the medium. The pH of the medium was set at 7 and the medium was micro-oxic. Sodium sulfide was added to scavenge the oxygen.

The enrichment cultures were diluted 2 times till 10^{-8} and the last tube with growth (10^{-12})was examined microscopically. This dilution was used as an inoculum for the chemostat. Growth rates were determined for both semi-pure cultures of AAF and BG by measuring the optical density with time. The products of the fermentation were determined in batch culture by HPLC

Two pumpcurves were determined prior to the chemostat build-up by measuring the outflow in milliliters against time at a constant pressure introduced on the tubing. Two chemostats were build up from autoclavable materials like seringes, needles, stoppers, tubing, glass, as shown in the schematic overview in figure 4. The chemostats and the bottle with medium supply were filled with medium without substrate and the whole setup was autoclaved. Before autoclavation, the medium supply bottle was equipped with a seringe filled with a plug of cotton wool for sterile aeration purposes. The tubing from the medium supply to the chemostats was closed with clamps. The waste collection bottle was aerated in the same way as the medium bottle. After autoclaving, the aeration seringes were removed and dextrose and Na_2S were added to the medium supply as well as the chemostats to complete the medium. The chemostats were inoculated with 10 ml inoculum from both AAF and BG in a total volume of 300 ml. The chemostats were operated at constant speed at a value equal to the growth rate. Samples were taken after 1, 2, 2.5, 3, and 3.5 days, analyzed on the HPLC, and microscopically examined.

Results and Discussion

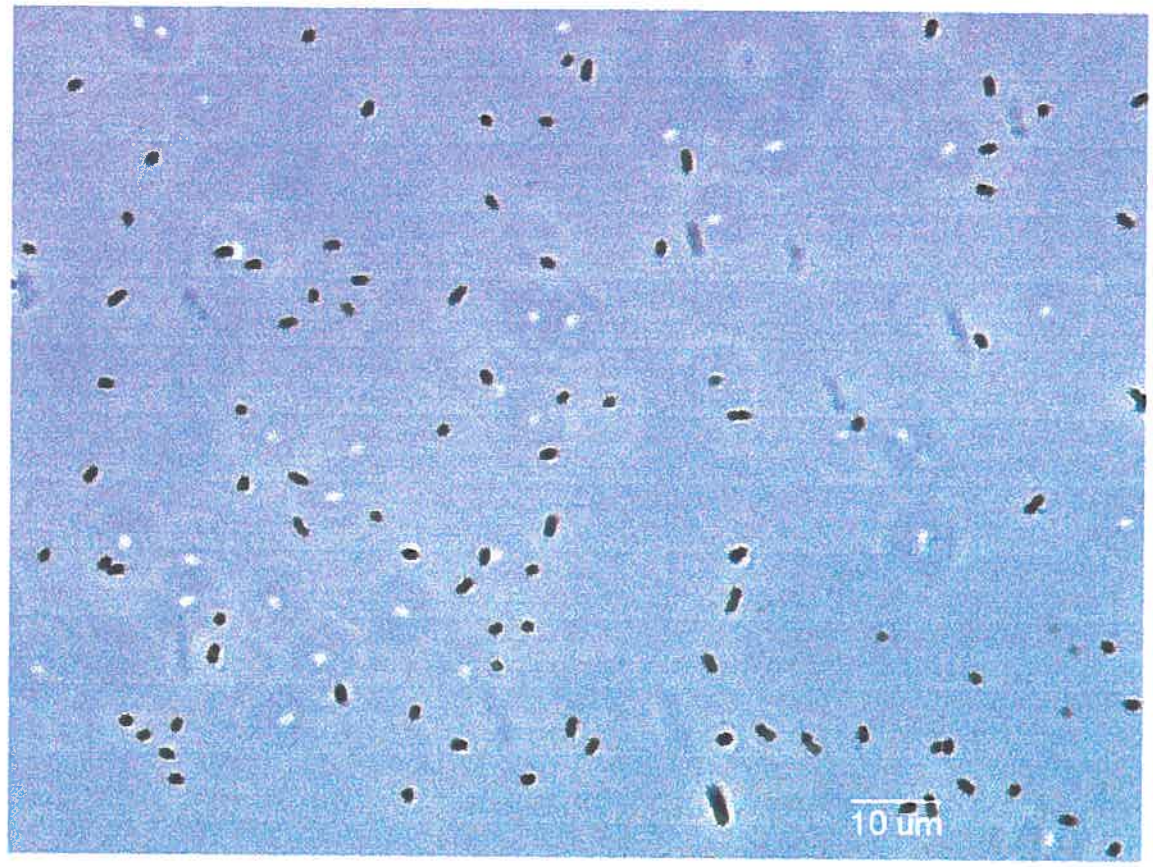
The 10^{-12} dilution of both AAF and BG were microscopically determined as no pure cultures, but at least consisting of 2 different microorganisms. AAF comprises small rods and cocci, whereas BG comprises long rods and two aligned rods (Figure 1). The two semi-pure cultures are distinguishable from each other by cell morphology. This makes them still useful for the chemostat competition experiment, although it would be easier to work with pure cultures.

AAF has a growth rate of 0.037 h^{-1} and BG a slower growth rate of 0.029 h^{-1} (Figure 2). To reach a steady state in the chemostat, the dilution rate should be set at the same rate as the growth rate. With growth rates of 0.04 and 0.03 h^{-1} and a volume of 300 ml this implies a flow rate of 0.18 and 0.15 ml/min. The pump was set at 0.4 giving chemostat 2 a flow rate of 0.18 ml/min and chemostat 1 a slightly slower flow rate of 0.14 ml/min, due to less pressure on the tubing.

HPLC results show that AAF ferments glucose to lactate and acetate and a compound eluting at the same time as pyruvate. BG produces only pyruvate and acetate (Figure 3). The compounds were identified by comparison with elution times of standard mixtures of acids and alcohols. The elution times of the standards, however, can be slightly different from the elution time of the same component in the sample due to matrix effects caused by different salinities and acidities in the samples. To be completely sure of the identification it would be better to spike the samples with a single known compound and use this elution time.

The chemostat and the medium supply bottle were pink after autoclaving (Figure 4), but the chemostats turned colorless after addition of sodium sulfide and stayed colorless during the full experiment what means that they stayed anearobic. The compounds measured in the 2 chemostats show a different pattern over time (Figure 5). In chemostat 1, the concentration glucose decreases and concentrations of acetate, lactate, and a unknown compound (most likely to be ethanol) increase over time. Pyruvate concentrations are stable. The decrease of the substrate glucose and the increase of the fermentation products indicate that the chemostat did not reach steady state yet. The pump speed was apparently to slow to initiate steady state conditions. In chemostat 2 the glucose concentration is low and stable. This is how the chemostat is supposed to run; steady state conditions and low substrate concentration. The microorganisms eat up all the glucose that is pumped in. The product concentrations, however, are more variable over time. This is probably due to variations in outflow of the chemostat. Both chemostats show that glucose is fermented and acetate, lactate, and ethanol is formed. It is not possible to calculate the stoichiometry of the fermentation reaction because the pH, H_2, and CO_2 were not determined.

On the last day of the experiment, samples were examined microscopically. There was no change observed in the population , both AAF and BG were still present in the chemostat (Figure 6).



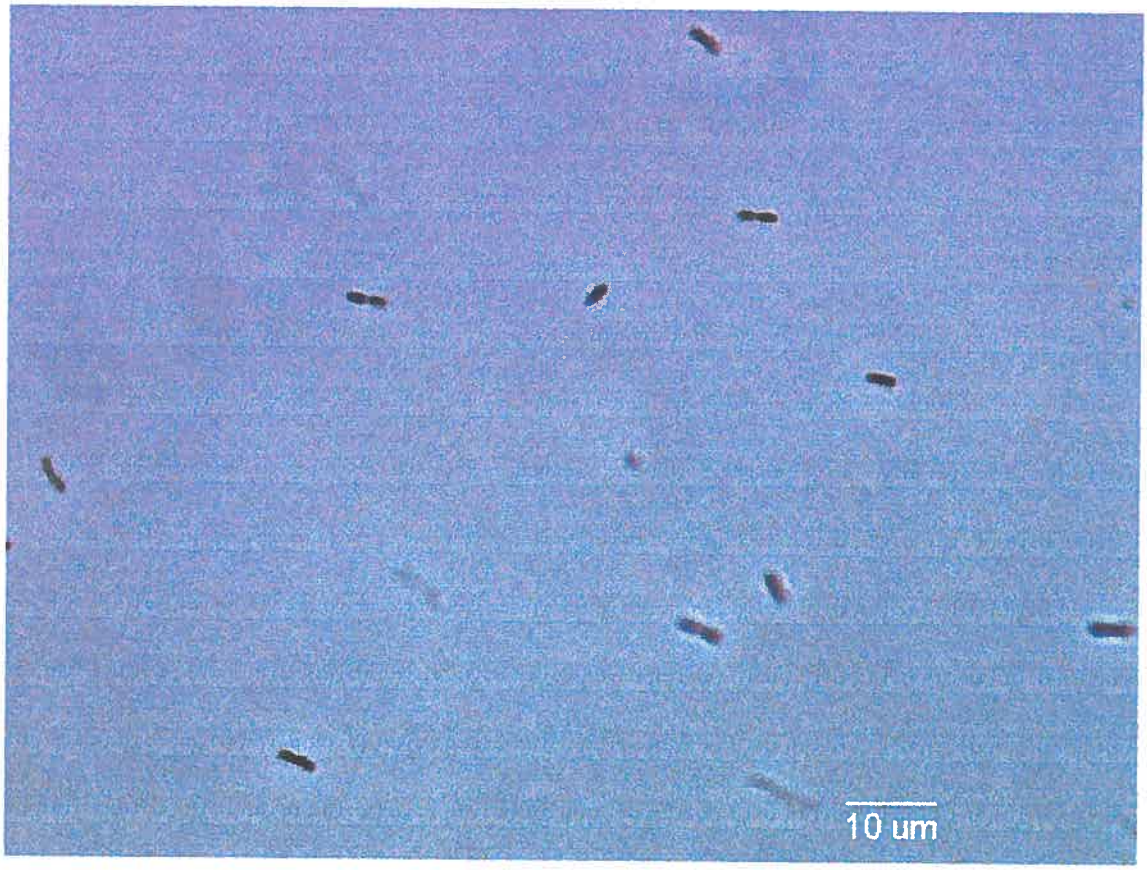


Figure 1: Semi-pure cultures AAF and BG from a batch culture

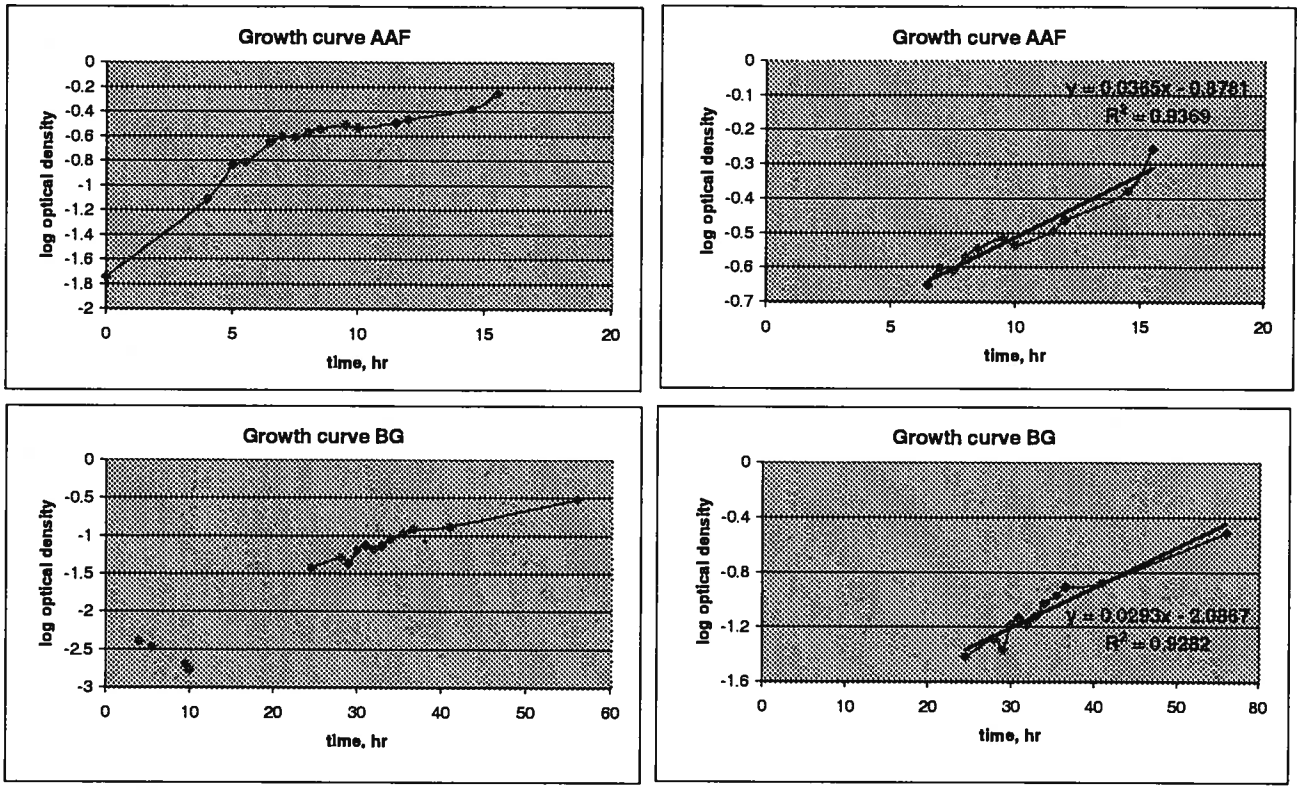


Figure 2: Growth curves of AAF and BG, with the slope of the linear regression line expressing the growth rate.

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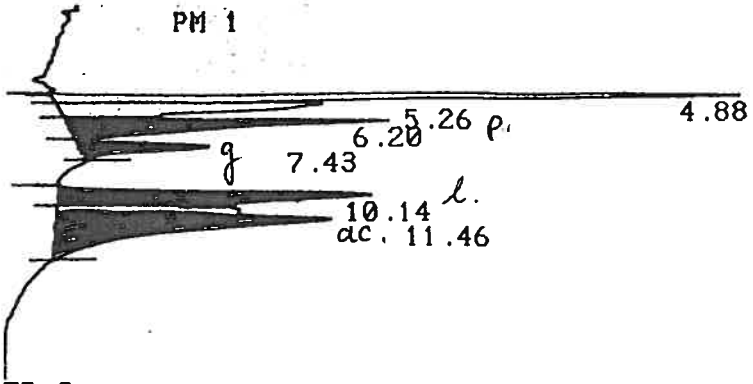

AAF

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PEAK#	AREA%	RT	AREA	BC	
1	17.173	4.88	266351	02	
2	12.381	5.26	192029	02	
3	15.401	6.2	238867	02	pyruvate
4	5.998	7.43	93022	03	glucose
5	18.993	10.14	294572	02	lactate
6	30.054	11.46	466138	03	acetate
TOTAL	100.		1550979		

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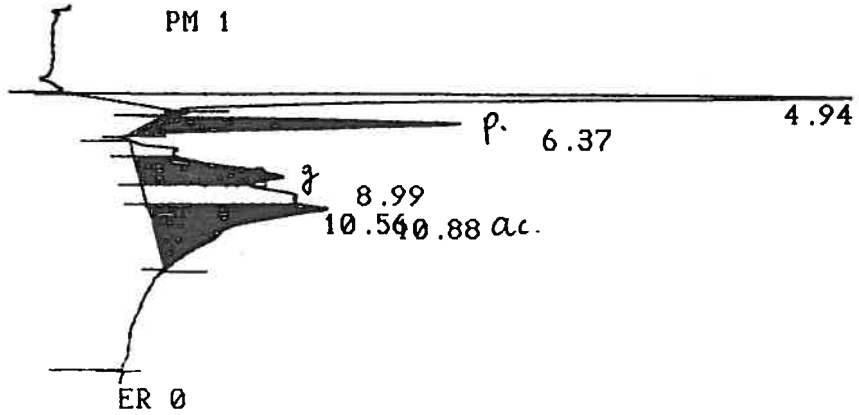

BG

DATA SAVED TO BIN # 75

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FILE 1. METHOD 0. RUN 203 INDEX 203 BIN 75

PEAK#	AREA%	RT	AREA	BC	
1	27.195	4.94	397929	01	
2	15.422	6.37	225660	01	pyruvate
3	2.221	7.92	32494	02	glucose
4	15.897	8.99	232611	02	
5	14.087	10.56	206130	02	acetate
6	25.179	10.88	368443	03	
TOTAL	100.		1463267		

Figure 3: HPLC-analysis of AAF + BG

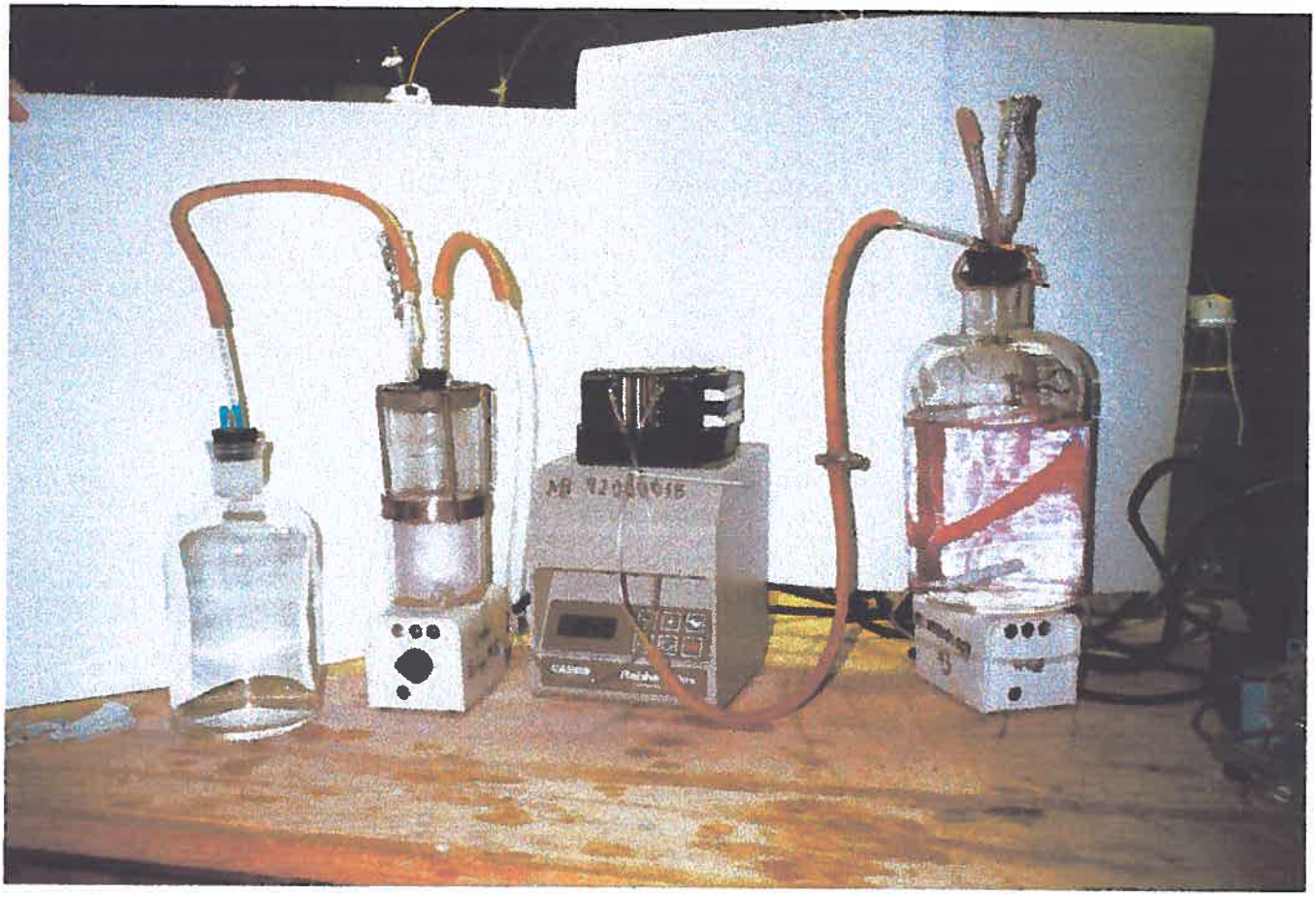
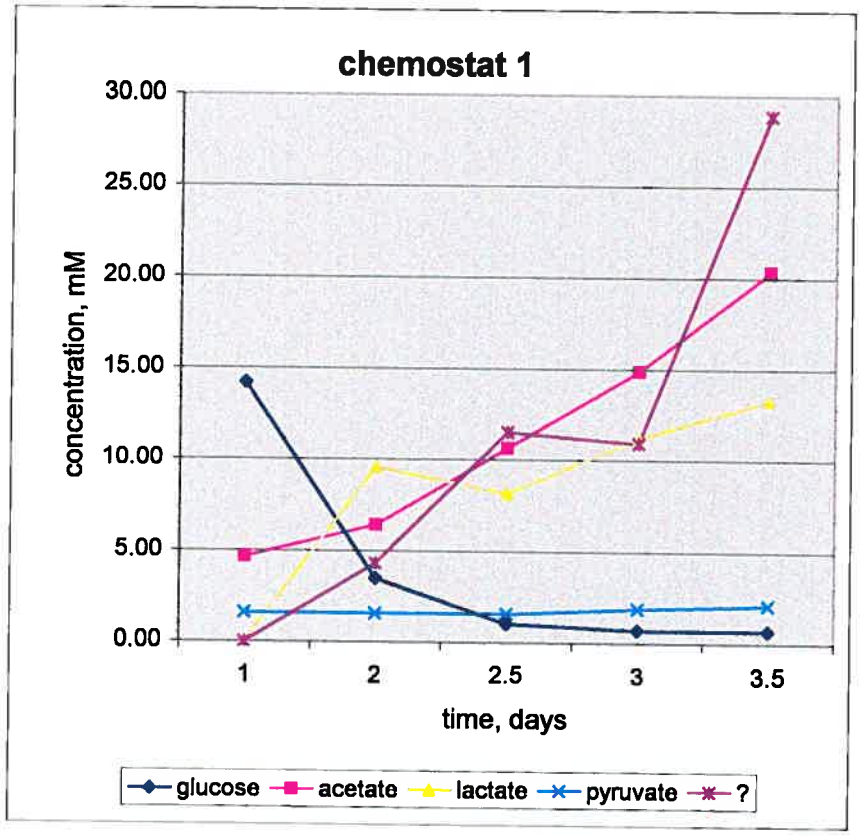


Figure 4: Chemostat setup



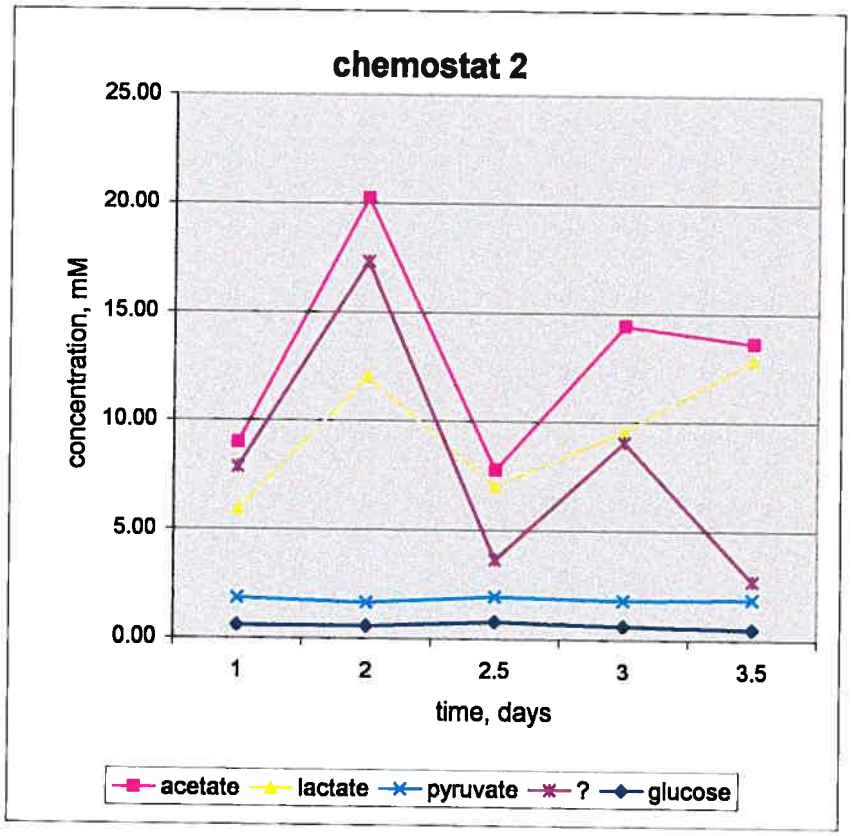


Figure 5: Substrate and products in two different chemostats

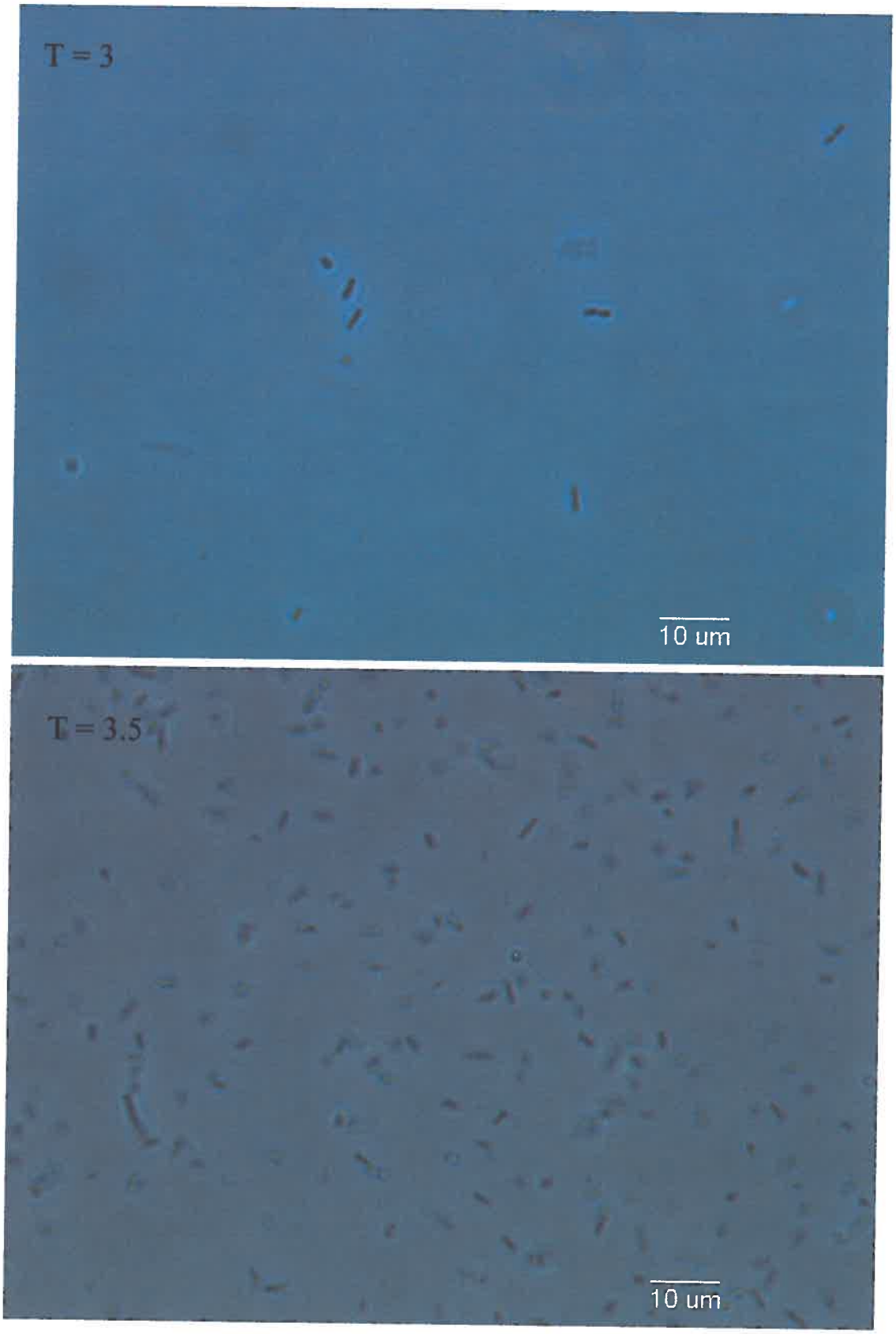


Figure 6: Chemostat culture sampled at day 3 and 3.5

Conclusion

Two semi-pure cultures were obtained from a casaminoacid rich plate and sludge from a zinc mine water treatment reactor. The cultures were named amino acid fermenter (AAF) and black gold (BG). The growth rate of AAF in a glucose medium was 0.037 h^{-1} and the growth rate of BG in the same medium was 0.029 h^{-1}. AAF was determined to ferment glucose in lactate, acetate, and pyruvate, whereas BG produced only acetate and pyruvate. In the chemostat the products appeared to be slightly ifferent. Pyruvate was only produced at very low concentrations whereas ethanol and acetate were the main products of fermentation. Chemostat 1 did not reach steady state because the inflow rate was too low. Chemostat 2 worked very well, started with a steady state from day one on and the glucose concentration stayed low and constant during the whole experiment. There was no change in population after three days. Probably it takes a lot longer to outcompete the slower growing organism BG.