

Forschungspraktikum

Production of volatile fatty acids through fermentation of paper mill sewage water

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Abbreviations and chemicals

Abbreviations

PHA	polyhydroxyalkanoate	
VFA	volatile fatty acids	
c	concentration	(mg/l)
TOC	total organic carbon	
PHB	polyhydroxybutyrate	
RT	retention time	(d)
PS	polystyrene	
PP	polypropylene	

Chemicals

N	nitrogen
P	phosphorous
NaOH	sodium hydroxide
HCl	hydrochloric acid
O	oxygen
CH ₄	methane

Abstract

To tackle the worldwide problem of plastic waste, new eco-friendly and economical profitable polymers have to be found. Polyhydroxyalkanoates (PHA) are a group of both bio-based and biodegradable polymers, which can be used to produce different kinds of plastic qualities. One way to produce PHA is to feed a type of bacteria found in paper mill sludge with volatile fatty acids (VFA). VFA can be produced by fermenting sewage water from the same paper mill. This step is researched in following article with the goal of maximum yield. In a laboratory scale the fermentation process was analysed with different level of pH (5, 7, 9) in a batch trial for 31 days and a continuous trial for 7 days. Visible changes, total organic carbon and dry mass was also noted during that period. The best outcome could be seen at pH 9 with a median growth rate of 12.61 mgVFA/day. The main component was acetic acid which can be used to produce polyhydroxybutyrate (PHB), which is an alternative to polystyrene (PS) and polypropylene (PP)

1 Introduction

The great pacific garbage patch, an island made out of plastic, which floats between Hawaii and California shows one of the biggest challenges in the modern world. With a size 4,5 times larger than Germany and an estimated weight of 79 thousand tonnes it is only the tip of an iceberg [10][7].

Straws, electronic devices, food packaging or clothes, those are only a few examples of products that contain plastic or are completely made out of it. Plastics make a lot of things easy nowadays due to their outstanding characteristics. Cheap production prices and the ability to adopt any shape and hardness seems to present an unlimited utility range. During the last century it entered our life and changed the way we use products in various number of ways. It revolutionised a lot of areas, e.g. the medicine, science and food industry. More people now have access to pharmaceuticals, fresh food and safe water all around the globe, which contributed positively to the wealth of the world's population. Furthermore, the demand for plastic products also increased [1]. The main resource of plastics are fossil fuels, which show a negative impact on the environment, because it used to be a carbon sink, but with the incineration of plastic products the carbon is released into the air and contributes to the increase of earth's temperature [8]. Furthermore, plastic doesn't degrade entirely, which seems to be a positive characteristic on first sight, because it has a long life span, but it also means that every piece of plastic that ends up in nature stays there. UV-light and rough surfaces can reduce larger plastic pieces to small pieces. Those are often eaten by animals, e.g. fish and find their path into our food chain [12]. The long term side effects of plastics on human bodies, which enter it orally, dermally or through inhalation are recently studied. Chemicals used to change the characteristics of certain types of plastics are suspected to have an effect on fertility for example [16].

A new movement, that occurs in countries all around the world tries to tackle the problem by producing less or even zero amount of waste, which is a great start to tackle the problem of waste, but plastic is still vital for most people, so scientists are trying to find new resources to produce plastic in a more sustainable way [11].

One way to produce a polymer, which is bio-based and biodegradable is covered in following report. Sewage water from the pulp and papermill Billerudkorsnäs in Gruvön, Sweden is used in two ways. Firstly a preparatory step in order to gain volatile fatty acids through a fermentation process can be carried out and is the main subject of following article. Afterwards the VFA rich solution is used to feed a polyhydroxyalkanoate accumulating bacteria, which was found in the sludge from the paper mill biological treatment plant [4]. Depending on the type of VFA fed to those bacteria the type of PHA and its qualities changes and subsequently be used to produce different plastic-like products [4].

The improvement of the fermentation step in order to gain VFA is depicted in following report. Various batch trials with different pH-values were carried out along with a continuous trial using the most successful samples.

2 Definition of task

The project deals with the fermentation step of a process to produce PHA from sewage water of a paper mill factory. Since paper mill sewage water consists of various kinds of organisms, carbon sources and lacks nutrients due to its dilution, it is hard to predict the outcome from start. First experiments have shown that there are bacteria, that are able to produce Volatile Fatty Acids, which is the desired product. Under anaerobic conditions three different trials with different pH levels were carried out and the VFA production observed. Highlighted were also the visible differences in order to gather more information about competing organisms.

3 Material and Methods

3.1 Sewage water

Approximately 5 l of sewage water was gathered at the Billerudkorsnäs paper mill in Gruvön, Sweden one day before the start of the experiment. It was collected from the fibre sedimentation step of the wastewater treatment. Therefore, it contains high amounts of organic material with origin from wood, such as larger and smaller fibres as well as soluble matter. Kraft pulp is used in the mill, which might cause an increased amount of sulphur in the effluent [5].

Due to the lack of nutrients, the sewage water was prepared by adding 0.24 g solid ammonium sulphate ($c(N) = 0.1 \text{ g/l}$) and 0.055 ml of 85 % phosphoric acid ($c(P) = 0.034 \text{ g/l}$) to 2.4 l sewage water [4]. Afterwards, the water was separated equally to adjust pH for each experiment by adding NaOH (1 M) or HCl (5 %).

3.2 Reactors and set up for batch trial

For the experiments 6 Schott flasks with a volume of 500 mL and a working volume of 400 mL were used. Three different kinds of pH-values were tested, so every experiment was performed as duplicates. The prepared water was distributed into the bottles in the following way and a mixed inoculum culture with bacteria of a previous fermentation of paper mill sewage water experiment and bacteria of a fermentation of jam factory sludge was added in the following way.

Table 1: Reactor set up for batch trial

Name	pH	Amount of liquid (ml)	Amount of inoculum (ml)
1.1	9.36	400	1.5
1.2	9.36	400	1.5
2.1	7.04	400	1.5
2.2	7.04	400	1.5
3.1	5.00	400	1.5
3.2	5.00	400	1.5

To keep anaerobic conditions, the flasks were closed airtight with a stirring attachment (micro motors HL 149 12.21). The coupling contained two tubes to take samples. Those were closed with household clippers. The flasks were stored in a 37 °C water bath and the stirring attachment was turned on.

3.3 Reactors and setup for continuous trial

On day 23 one flask of the pH 9 and pH 7 batch trial were used to start a continuous trial with the leftover pulp and paper mill sewage water, which has been stored in a fridge. A Gilson minipuls 3 motor was used on the lowest setting (= 0.5). Parafilm was used to close the openings of all flasks.



Figure 1: Set up continuous trial

The final set up differs from the picture. The bottle with the sewage water was split up into two bottles and pH was adjusted to pH 7 and pH 9.

3.4 Measurement

A syringe was filled with N_2 (g) and afterwards injected into the bottles through one of the tubes. The N_2 (g) caused a pressure that pushed out a volume of sample out through the other tube. The sample was collected into a 15 ml disposable centrifuge tube. The goal was to prevent O_2 flowing into the flasks and to keep anaerobic conditions. Samples were taken every day from Monday to Friday.

3.4.1 pH-measurement

PH was measured on every sample using a pH electrode. The electrode was calibrated every day. To keep equal conditions during the experiments, the pH was adjusted if needed with NaOH (1 M) or HCl (5 %).

3.4.2 TOC-measurement

TOC was measured at the start of the experiment and after that once a week. A Hach LCK 387 measuring kit was used.

3.4.3 VFA-measurement

The VFA content was alternately measured on one of each duplicate. On day 17 and 20 VFA content was measured on all samples after differences in the progress of the reaction could be seen. A Hach LCK365 analyse kit was used.

3.4.4 Measurement of dry mass

Dry mass was measured by drying the samples which were not used for the VFA-measurement in an 100 °C oven over night. The proportion of dry mass to liquid was then calculated using following equation.

$$\%_{dry} = \frac{m_{after} - m_{before}}{m_{liq}} * 100 \quad (3.1)$$

4 Results and Discussion

4.1 Batch trial

The batch trial to gain VFA from paper mill sewage water was running for 31 days. The measurement of pH was carried out every day and was adjusted if needed. VFA content and dry mass was measured every second day and TOC was measured once a week.

4.1.1 Visible differences

Colour wise, the flasks with pH 9 turned out the darkest during the whole experiment. The colour was dark grey. The flasks with pH 7 were lighter grey and the flasks with pH 5 were more light brown. After three days a sulfuric smell was noticeable in the pH 7 trials and after 14 days white flakes started to form in the same batch. H₂S is a common by-product in anaerobic fermentation and the results indicate, that pH 7 presents optimal conditions for sulphur reduction. This development initiates the growth of *Beggiatoa*. Bacteria from the genus *Beggiatoa* can oxidise sulphide in order to accumulate sulphur intracellular, which leads to a white coloration. Those white clumps could only be found on the water surface due to their metabolic pathway. *Beggiatoa* can use oxygen as electron acceptor, which could get into the flask while taking samples. *Beggiatoa* was only found in natural waters, which explains why it only appeared in the pH 7 trial [6][13]. Additionally, an increasing amount of gas was noticeable within the pH 5 trial on day 14.

4.1.2 pH

The pH was observed every working day. It is an indicator for VFA production, because fresh VFA lowers the pH due to its acidic character and former experiments showed that pH plays a major role in the outcome [3]. Figure 2 shows that the duplicates behaved similar in that category. The pH had to be adjusted between one and three times during the period in order to guarantee alternation of ± 0.3 . A prominent change could be seen during the first six days on the pH 9 trial with a change of pH of up to 1.4 units.

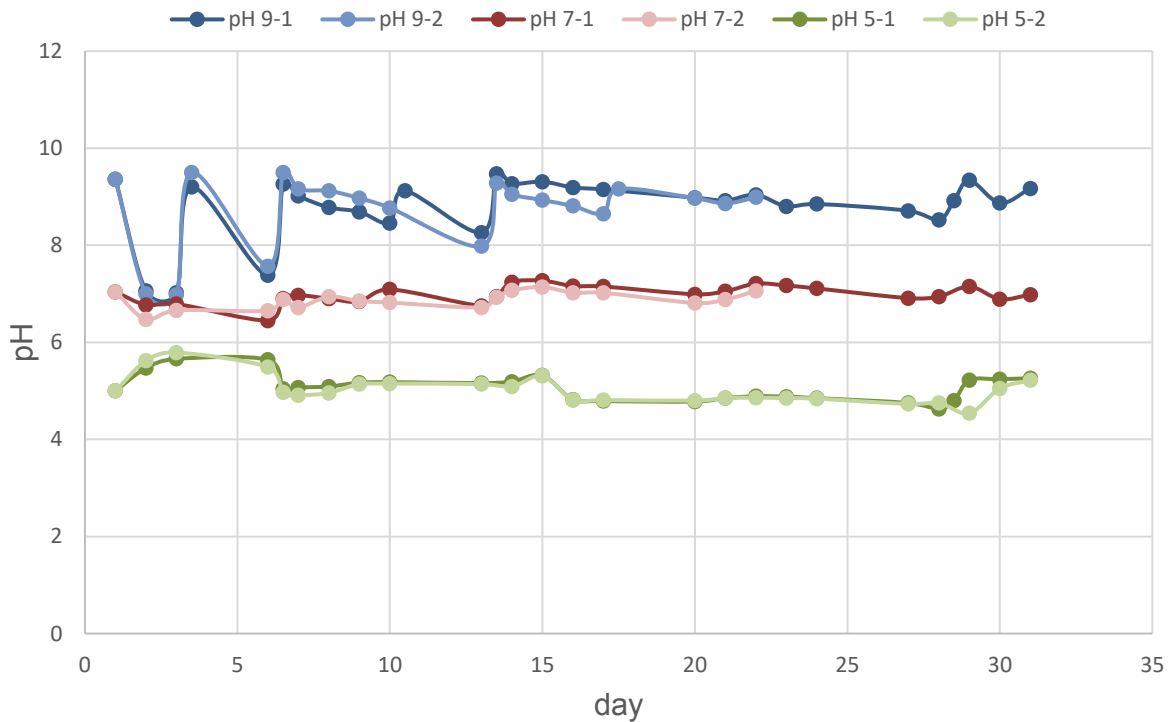


Figure 2: pH change over time for the batch trial

4.1.3 VFA

VFA content was measured alternating between one of the duplicate every second day. Overall, the graphs are showing a similar pattern for each duplicate and indicates an even development similar to the pH analysis. All trials could gain VFA constantly except during the weekend. The water bath turned off if there was not enough water in it, which happened during the weekends even after it was prevented by covering it with aluminium foil. A lower temperature inhibits the growth of VFA producing bacteria. However, clear results could still be seen. The median growth rates were 12.62 mgVFA/day for pH 9, 8.94 mgVFA/day for pH 7 and 6.51 mgVFA/day for pH 5. A maximum of VFA production was not reached after 31 days, but it can be said that pH 9 is out of the tested values most suitable for VFA production.

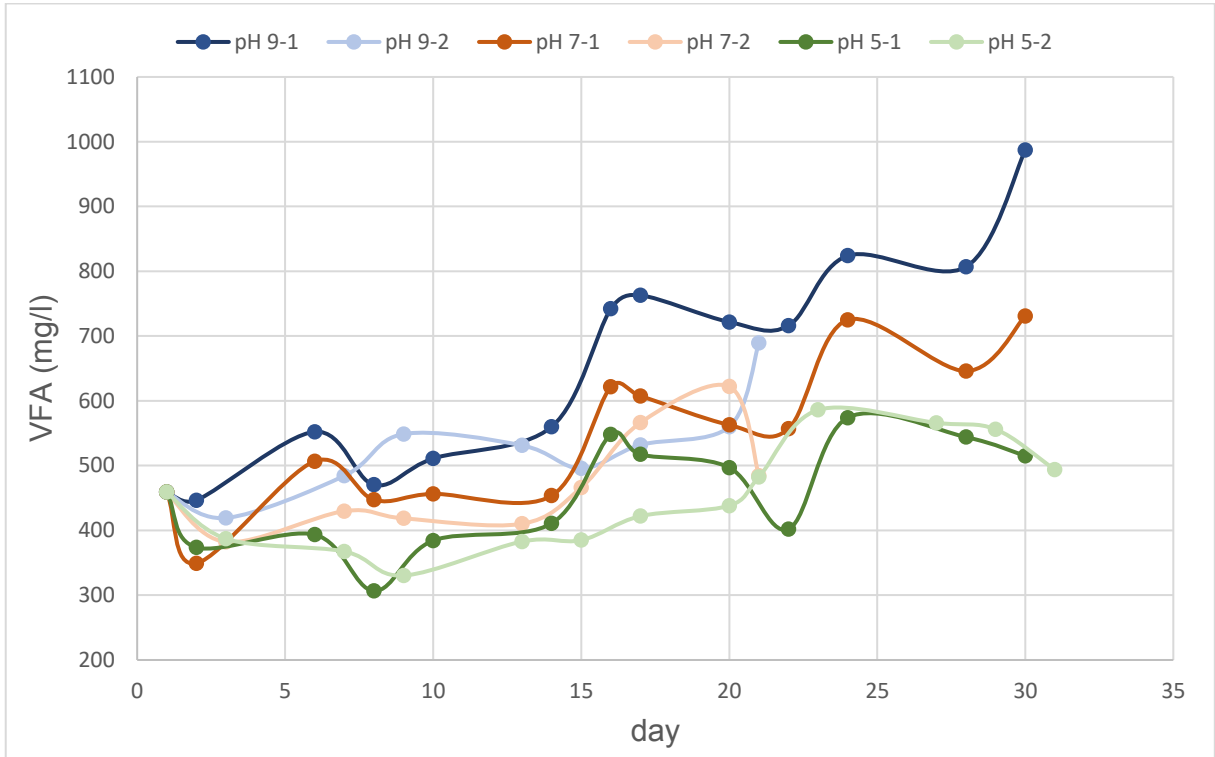


Figure 3: VFA change over time for the batch trial

A decrease of volatile fatty acids, which can be seen during the first days for all samples and especially for the acidic trials can be deduced to an increase of methanogenic bacteria. VFA plays a major role in the metabolic pathway of methanogenic bacteria [19].

A general lower VFA concentration for more neutral and acidic pH can be inferred to methanogenic bacteria since their activity is inhibited at alkali pH (>8) [18]. Furthermore, methanogens are also found in the rumen and can also be active in acidic pH [9]. At the same time Atasoy, M. et al. have previously shown how pH can change the outcome in terms of VFA production and had similar conclusions. A more alkali pH contributed to a higher yield [2].

An analysis of the VFA composition was carried out by Eurofins Environment Testing Sweden AB in the end of the trial shown in following table.

Table 2: VFA composition analysis

pH	Acetic acid (mg/l)	Propionic acid (mg/l)	Butanoic acid (mg/l)	Iso-pentatonic Acid (mg/l)
5	380	30	11	<10
7	500	<10	<10	<10
9	670	48	18	12

Iso-butanoic acid, pentanoic acid, hexanoic acid, iso-hexanoic acid and heptanoic acid were also measured. The concentration amounted to less than 10 mg/l and are not mentioned in the table. The main component found is acetic acid which can be converted into polyhydroxybutyrate afterwards [4].

The even development for each trial and the rising growth rate indicates that a higher pH is more suitable for the VFA outcome. In this experiment pH 9 was most successful and highest values of VFA were reached in the pH 9 reactors. A major role plays the degeneration of VFA through other organisms, especially with a lower pH which is inhibited due to the higher pH in the pH 9 trial. For more precise results more research with triplicates are recommended.

4.1.4 Dry mass

Dry mass was measured alternating between each one of the duplicates on every second day and calculated with the formula 3.1. It was used to estimate the amount of grown bacteria. Figure 4 shows a consistent low amount of dry mass until day 15 in all samples. One sample of pH 9 and pH 7 had a peak in dry mass on day 21. pH 5 showed a peak on day 23. Since one flask of pH 9 and pH 7 was used in the continuous trial there is no evidence about the validity of the peaks shown. The peaks also dropped sharply during the following measurement. Unexpectedly, dry mass did not grow in a similar way with the growth of the VFA concentration.

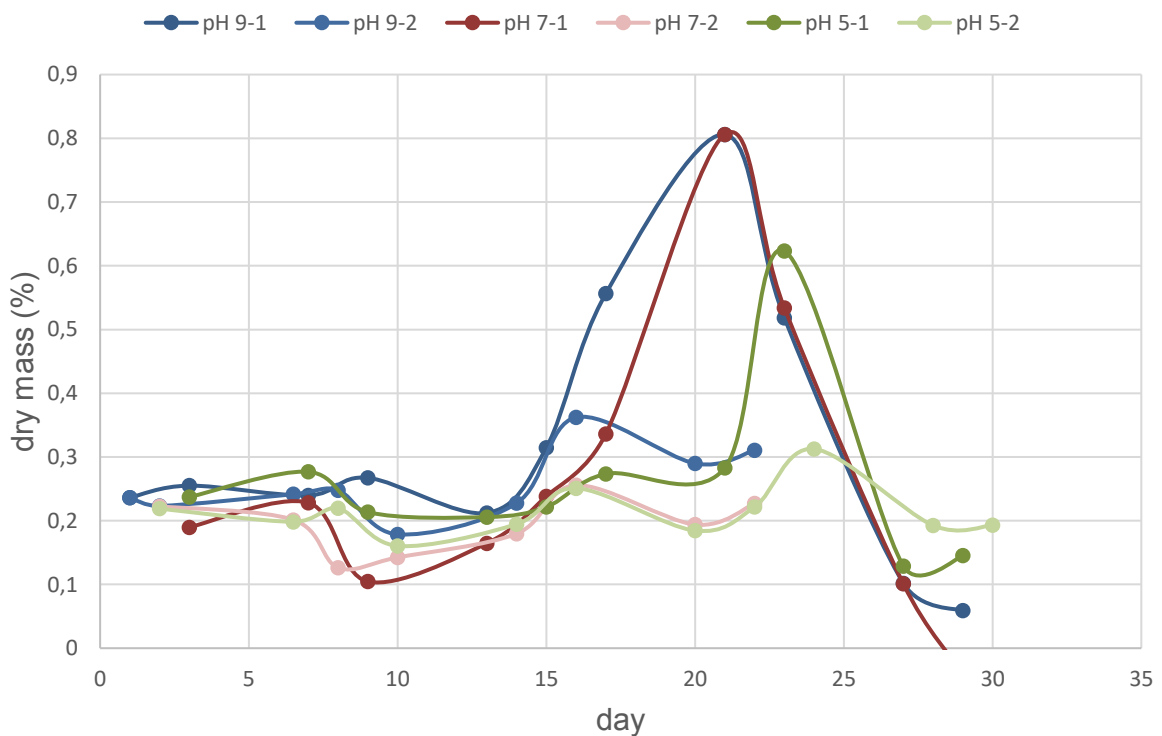


Figure 4: Alternation of dry mass for the batch trial

Since the result of the dry mass analysis deviates from the previous results and each duplicate behaved differently from each other, more research with triplicates are recommended.

4.1.5 TOC

The amount of total organic carbon was measured every week and was used as an indicator if any carbon was turned into gas. Representing for all experiments only one duplicate is shown in following figure.

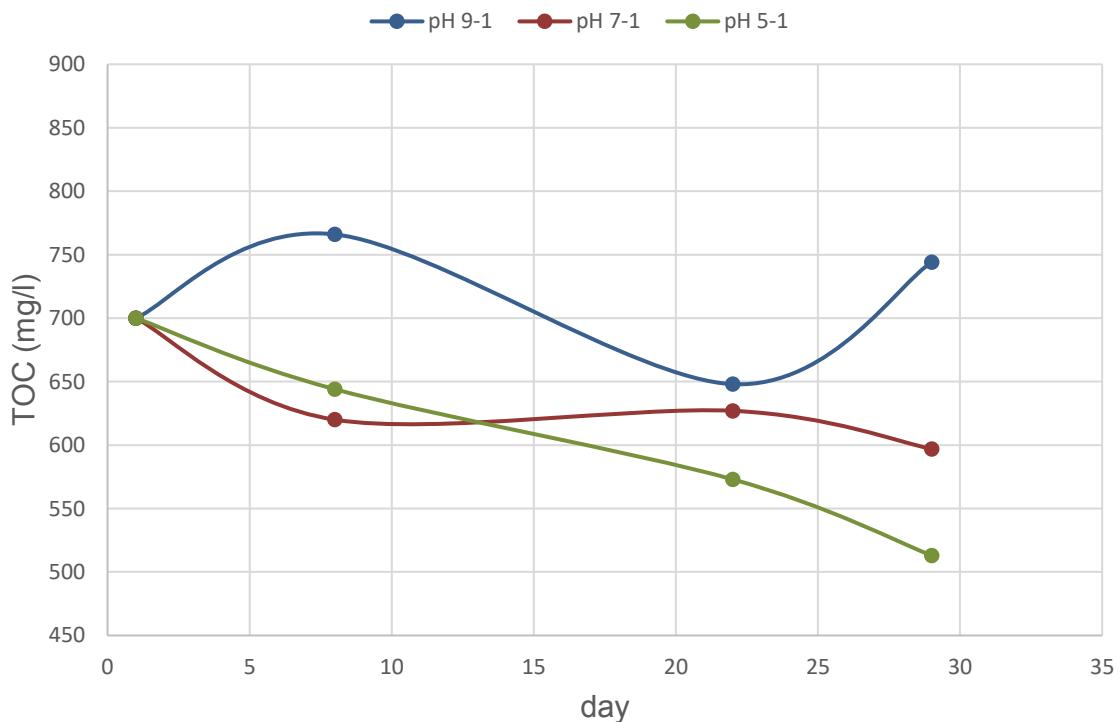


Figure 5: Development of TOC in the batch trial

The amount of TOC stayed high in alkali pH, whereas the other trials show a decrease in TOC since week one. The pH 5 sample differs from the pH 9 sample by almost 250 mg/l. During fermentation processes CH_4 (g) was expected from methanogenic bacteria. The decreasing amount of TOC confirms the hypothesis of the growth of methanogenic bacteria in lower pH.

4.2 Continuous trial

The continuous trial was carried out for seven days (162 h) with one sample of pH 9 and pH 7.

Table 3: Overview of the continuous trial

Sample	Used liquid (ml)	Total time (h)	Flowrate (ml/h)	Volume (ml)	Retention time (d)
pH 9	800	162	4.94	350	2.95
pH 7	955	162	5.90	350	2.47

4.2.1 VFA

VFA was measured every workday. It decreased during the first 5 days, probably because some of the original water was flushed out. After five days an increase of VFA could be measured. Due to the lack of time and sewage water the experiment could not be carried out longer and valuation of the data is not possible.

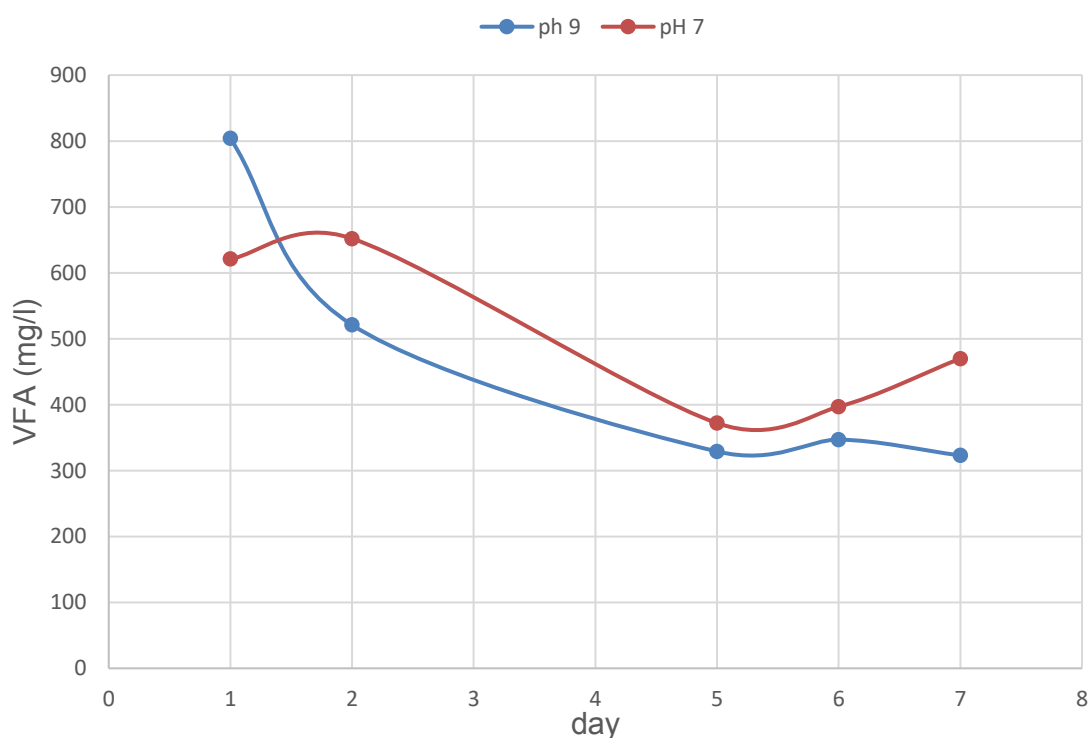


Figure 6: VFA change over time for the continuous trial

Previous experiments from Simon Bengtsson et al. [3] have used the same type of waste water. The continuous trial shows an increase of VFA with increase of retention time. Since the VFA production in the present case showed its peak after 16 days, a longer RT was used. However it seems that it takes two full cycles until VFA concentration increases.

5 Conclusion

Plastics are a vital resource in current times. However, it is still primarily produced using fossil oil, which contributes to the environment in a negative way. Working with resources that already exist, like paper mill effluent in order to produce polymers is a great way to enhance the ecological footprint of plastics.

This report deals with the production of volatile fatty acids made out of fermented paper mill sewage water, which can be used to produce PHA, a bio-based and bio-degradable polymer. The goal of this work was to find out how to optimize the VFA production.

Therefore three different types of pH were used. The development of pH, VFA and TOC concentration and the dry mass over a period of 31 days was analysed in batch trials. Best results could be seen at pH 9 with a VFA growth rate of 12.61 mgVFA/d and 1090 mg/l of VFA on day 31, but further growth can be expected. The pH 7 trial showed a growth rate of 8.94 mgVFA/l and the pH5 trial 6.51 mgVFA/l. Methanogenic bacteria, that grows better in neutral and lower pH seems to be the main reason why VFA concentrations were higher at an alkali pH. Methanogens produce methane while depleting volatile fatty acids. At pH 7 there was also a growth of *Beggiatoa* noticed. A VFA analysis showed, that there was a predominantly amount of acetic acid, which can be transformed to PHB in a following step, that is not covered in this work [4]

A continuous trial with two different samples of the batch trial were started on day 23 and has shown an increase of VFA after 5 days. Indeed, more research has to be done in this field to create more valuable results.

The production of PHB has a lot of advantages. On one hand PHB has similar properties like polystyrene and polypropylene [15]. PP and PS are often used as food packaging. Whereas PP is suitable as foil, because it is moisture and fat repelling, PS is used as packaging for meat or vegetables as well as container for to go food [17]. Those products cannot be left out in modern life due to its convenience and ability to transport food safely. Even so it has economic advantage as well. Research from Sandberg, M. et al. showed that PHA production along with hydrogen production can gain up to 40-100 million SEK or 4-10 million EUR per year for a paper mill in Sweden. It was assumed that the PHA production can yield up to 2000 tons per years [14].

6 References

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