Thesis Biobased Chemistry and Technology

Conversion of PHB in cells into methyl crotonate as first step to obtain biobased acrylates and propylene

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Conversion of PHB in cells into methyl crotonate as first step to obtain biobased acrylates and propylene

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1. Abstract

Polyhydroxyalkanoates (PHAs) are biopolymers that can be stored in some specific microorganisms during a fermentation process with the use of cheap substrates like sucrose, agro-industrial residues and activated sludge. The most important type of PHA is Polyhydroxybutyrate (PHB) which has been produced on pilot scale. [1]

Nevertheless, PHB as material has shown several drawbacks compared to conventional polymers like stiffness, brittleness and its poor thermal stability at temperatures close to its melting point (176°C to 188 °C). These drawbacks prevent them from substituting the fossil based polymeric materials in commercial products. [2]

The aim of this master thesis will be the study of the first part of the reaction from PHB to methyl crotonate (MC) without the need of the expensive purification process using PHB directly from fermentation without any further upstream processing apart from drying.

Reaction conditions of 200°C, 13 bar and 6h, adding 10 mL of methanol to obtain the ester from 0.6 g of PHB results in a 49% conversion of MC while with pure PHB (53%).

It has also been tested that reaction with the samples of PHB taken directly from the fermentation stage shows a faster reaction than with pure PHB reaching the optimal conversion of 48% in 3h instead of 6h.

2. Introduction

Petroleum-based plastics show versatility and low cost, and they can also be used as substitutes of wood, metal...

Plastics are actually a derivative of petroleum, natural gases or other fossil resources. As a petroleum product, plastic contributes to oil dependency, at a time when it is generally recognized that oil will not be available indefinitely. The use of petroleum in its production causes a huge environmental problem because the global carbon cycle is being destroyed, while for biomass/bio-organic compounds it takes 1-10 years to turn to CO_2, fossil resources petroleum and natural gas turns to CO_2 in more than 10^6 years.

Plastic waste generated by industries require up to hundred years for total degradation [3]. An approach to decrease the solid waste [4] can be to substitute conventional material with biodegradable raw materials to reduce costs and to enhance the degradation of the final product.

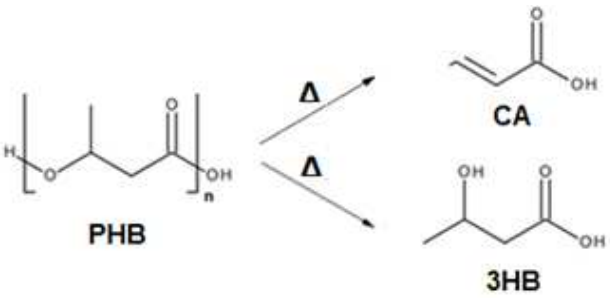
Over time plastics actually photodegrade into smaller and smaller toxic pieces but never disappear. Many of these tiny pieces end up in our oceans and waterways and are eaten by marine life. The toxicity of plastics is not fully understood or adequately tested. Most plastics contain chemical additives to make the plastic more pliable, or UV resistant, etc. Some of these ingredients or additives are not thoroughly tested [5].

Bio-based material production has become one of the most important researches due to limit supply, increasing price of crude oil and demands for environment sustainability. The last studies [6] show that PHB involves less complicated steps, high yields and the use of cheaper substrates.

Poly(3-hydroxyutyrate) (PHB), is a bio-polyester produced by many bacterial species as an energy storage material, it has material properties similar to petrochemical polymers like polypropylene for a variety of applications [7].

PHB was discovered in 1926 but its use was overlooked because petroleum was cheap and abundant [8]. Commercialization of PHB is difficult due to high production costs and also because PHB shows some drawbacks like its fluctuating properties and the low thermal stability [9] The processing conditions for PHB have been reported to be from 170°C to 200°C, which are really close to the temperature where degradation of PHB can be observed and also because it has an expensive purification process. [9]

PHB degradation has been studied before in order to obtain crotonic acid (CA) and 3-hydroxybutyrate (3HB) through a pyrolysis process [2] as it can be seen in scheme 1. Without the use of catalyst [6] 63% CA can be obtained at 310°C from PHB degradation and it can be converted to acrylic acid and propylene via metathesis with ethylene, which are important products for the plastic industry [10.]



Scheme 1. Degradation of PHB into CA and 3HB.

Nevertheless, the temperature used for the degradation process of PHB is high and also as CA is soluble in water its purification process is complicated. It has been researched in previous publications that another conversion is a PHB transesterification process in presence of acidic methanol or butanol to form 3-hydroxybutyrates and also that when base or more than 60% was used for the assay, crotonates were formed from methyl 3-hydroxybutyrate (3HBMe) [11] .

For now, a transesterification to produce methyl crotonate (MC) from PHB is unknown so far and it can also be used as a substrate to obtain biobased propylene and methyl acrylates. Methyl crotonate is insoluble in water so the separation stage would be easier and also it can be used to obtain biobased propylene and methyl crotonate. As far several experiments have been done with purified PHB [10], in presence of methanol in a single step, without the use of catalysts.

Purification process of PHB has less complicated steps than petroleum plastics production (Figure 1)[13] and also uses cheaper substrates than the petrochemical way like activated sludge, starch, whey cheese, glucose...

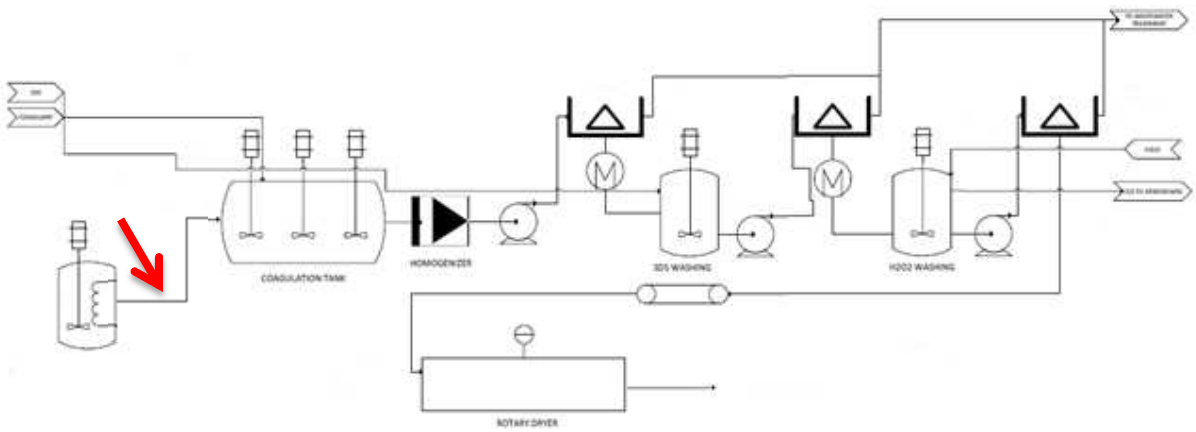
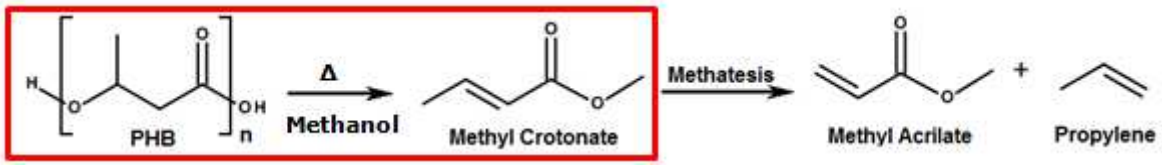


Figure 1. Diagram Scheme of purification process of PHB. Red arrow indicates the point of extraction of samples PHB Cells.

As it can be seen in figure 1, PHB is purified through several treatments to dry it and also to remove all the biomass present in the streams that come from the reactor, which involves high energetic requirements for the centrifugation and treatments to remove the biomass.

The aim of this thesis will be finding a solution of these drawbacks to use biodegradable polymers to obtain other useful chemicals like propylene or methyl acrylate, which have a lot of applications in adhesives, chemical intermediates, coatings, textiles and plastics. The substrate used in this case is waste water from Mars Bars factory in Veghel. PHB was taken directly from the fermentation process (Figure 1, red arrow) skipping the purification process, to remove biomass formed by the rests of cells used in the fermentation and also nutrients from the fermentation stage. The process with pure PHB has been already studied in our group, using 200°C, methanol as a solvent and pressures from 15bar and 20bar, all these conditions provided an optimal conversion of methyl crotonate .

The samples of PHB Cells used in all experiments were provided by Delft University of Technology. The reaction that will take place is shown in the following scheme:



Scheme 2. Chemical conversion of PHB to methyl acrylate and propylene

Once the amount of PHB in the feed is known, the sample will be examined through a TGA (Thermogravimetric Analysis) to know the onset degradation temperature of PHB and the maximum one. The experiment will be tested with different temperatures between the onset and maximum degradation temperatures.

The first experiments will focus on the idea of using samples of PHB Cells in the reaction using the same conditions that were used in the reactions with pure PHB. The standard conditions that has been settled are 200°C, t=6h, P=15-20 bar, 10 mL solvent (methanol)

The weight losses present in the TGA until 110°C were referred to the removed water from the biomass and at temperatures from 110 - 170°C there would be a decomposition of bacterial cell components of the bacteria.

TGA is a technique in which the mass of a substance is monitored as a function of temperature or time as the sample specimen is subjected to a controlled temperature program in a controlled atmosphere.
The mechanism consists of a sample pan that is supported by a precision balance. That pan resides in a furnace and is heated or cooled during the experiment. The mass of the samples is monitored during the experiment. A sample purge gas controls the sample environment. This gas may be inert or a reactive gas that flows over the sample and exits through an exhaust.
TGA can also be understood as a technique in which, upon heating a material, its weight increases or decreases.

When a sample is heated in TGA, it releases volatile materials or generate combustion components as it burns. According to the experiments done by Eindhoven University of technology in an IR cell, the untreated biomass from Mars pilot plant will be the as follows in figure 3:

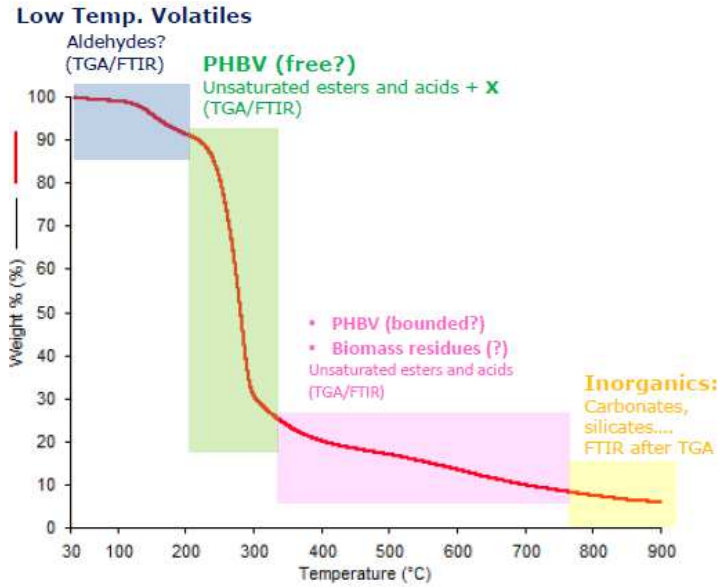


Figure 2. TGA of untreated biomass. TGA provided by Gizella Malkova from Eindhoven University of technology.

As it can be seen in Figure 2 , in the first part of the graph it is shown the exact composition of the volatiles produced during the degradation process.

In the last part of the graph shown in Figure 2, it can be seen that there are some inorganic elements produced from the degradation of the biomass. Another FTIR was done under nitrogen conditions to know the type of inorganics that are produced after the degradation. The results are as follows:

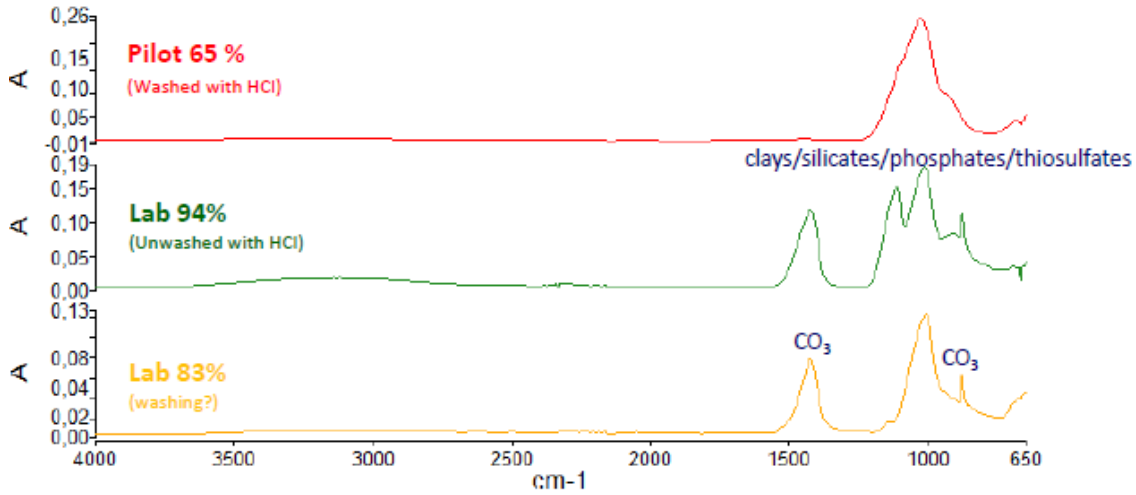


Figure 3. FTIR on sample decomposed in TGA (N_2). Provided by Gizella Malkova from Eindhoven University of technology.

As it can be seen in Figure 3, the samples from the lab contain carbonates and materials which could be related to silicates, phosphates or thisoulfates. The carbonates could be removed by HCl washing and it's known that inorganics can contribute to thermal degradation and/or degradation.

In previous work in our group [12] , the GC-MS analysis showed that during the degradation process of pure PHB some products like propylene and ethylene are produced, these are

typical products from degradation and come mostly from the decarboxylation of crotonates [8].

The experiment will also be done at different pressures. A certain amount of N_2 will be used to reach the desire pressures in the reactors, it is also important to know that during the reaction the methanol will turn to gas due to the high temperatures so it will also have some effect in the pressure that is reached in the reaction.

The samples used come from the fermentation process so they contain biomass and also huge amounts of water. The samples will be dried in order to remove all the water present before being used in the reactors, the samples will be put in a freeze drier and then in an infrared drier to know exactly the state of the samples in terms of dry matter and water.

There will also be experiments with different amounts of water to see the influence of water in the production of MC and also water/PHB.

The residence time for the reaction will also be studied to see if the degradation of PHB is the same as with pure PHB. The optimal residence time for the reaction of pure PHB has been settled at 6 hours [12] getting a conversion of 54% MC.

The presence of residues from the fermentation as biomass and nutrients from the cells will also be. The presence of some nutrients from the stock solution like K^+, Mg^{2+} used during the fermentation process will also be studied to see if it has any influence in PHB conversion. The initial stock solution used in the fermentation process in the pilot plant from Mars [1] contained also ammonium, but the samples were taken after depletion of the ammonium, so there is no NH_4^+. It will also be tested if the presence of these residues have any effect in the reaction, making the reaction go faster

The samples used from PHB Cells also contained a percentage of valerate of 16% while in pure PHB there is only 2%. Polyhydroxybutyrate-co-β-valerate (PHBV**)** is a copolymer of PHB containing segments of hydroxyvalerate, and like PHB is biodegradable, nontoxic, biocompatible plastic produced naturally by bacteria. It is a thermoplastic linear aliphatic polyester. A sample of PHBV with 20% Valerate will also be used during the experiments.

The structures of both polymers is very similar, with PHBV having an extra methyl group (Figure 4)

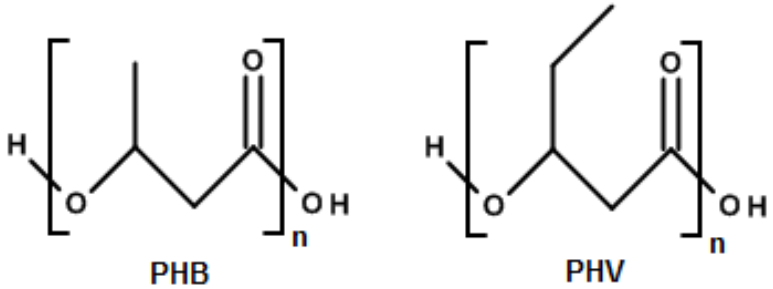


Figure 4. Structures of PHB and PHV.

3. Materials and methods

PHB was provided by Technical University Eindhoven (2mol% Polyhydroxyvalerate (PHV), Mn=450kDa and Mw/Mn=1.3)/ PHBV20 (20% valerate) was provided by ShanDong TianAn Chemical Co., LTD, Methanol HPLC gradient was provided by Actu-All Chemicals and $Mg(OH)_2$ bought from Sigma Aldrich.

The samples containing PHB directly from the fermentation process have biomass with high PHA content, these samples were conducted with wastewater from a candy bar factory (Mars, Veghel, The Netherlands) and had different compositions of PHB: sample A (16-10-2013, (54%PHB), sample B (22-10-2013, 51%PHB), sample C (26-10-2013, 50%PHB) and sample D (11-12-2013, 59%PHB).

The samples used for the experiments were put in a freezer to reach -200°C and then put in a freeze drier Christ Alpha 2-4 LD to remove all the water from the fermentation process. The samples were then put in an infrared drier to know the amount of water that was still present. After that, the samples were put in vials with a certain amount of methanol and then in the reactors, the reactors used were a Parr Series 5000 Multiple Reactor System.

The degradation temperature was studied through a TGA analysis. The reactors were taken to the reactor setup where temperature, pressure and residence time of the reaction were controlled with a software and it was also used to reach the pressure desired for the reaction software. The methanol present in the reaction turn to gas during the reaction, which made the pressure of the reactor increase and to get higher pressures it was also used nitrogen during the reaction. Before the reaction took place the system was purged with nitrogen and temperature was reached with a cooling system based on water

Once the reactions were done, the samples were taken with syringes and through filters were put in 10mL vials. The samples were diluted in a mixture with methanol (100µL sample + 900µL methanol) using micropipettes and then put in a HPLC to know the exact composition of crotonic acid, methyl-3-hydroxybutyrate, 3-hydroxybutyrate and the product methyl crotonate.

4. Results and discussion

Initial experiments

The first experiments will focus on the behaviour of the reaction with pure PHB to know the conversion and the reference values to do next experiments.

In Table 1 (entry 1) it can be seen that the conversion reaches 54% MC. Comparing to previous experiments done in our group [12] , the entry 2 shows that the conversion of MC is quite similar in both experiments while the rest of the starting materials (crotonic acid (CA) and methyl-3-hydroxybutyrate (3HBMe)) show very low conversions. There are also some losses in the system which are basically gases like CO or CO^2 and propylene originated from a decarboxylation reaction, reported for CA [32]. The pressure couldn't be calculated due to some problems with the pressure sensor during the experiment.

Table 1. Results from experiment JHO01, pure PHB.

Entry	MC (%)	CA (%)	3HBMe (%)	Losses (%)
1	54	3	8	35
2	53	7	12	28

Reaction conditions: 0.600g pure PHB, T = 200°C, t= 6h, 10 mL methanol. No 3HB (%) was observed. Average of duplicate experiments.

Once it is known the results with pure PHB, the same experiment in the same conditions will be done with samples taken from the outlet stream of the bioreactor for the production of PHB without any further treatment, which means that the product still contains biomass, water and according to the data from Delft University of Technology: acetate, propionate, butyrate, valerate, hexanoate, ethanol... (See appendix: PHA content and substrate (cells Delft university)). These samples were taken at different dates so they all have different compositions and delivered by Delft University of Technology, who obtained them from a pilot plant at the Mars factory in Veghel [1]. From here on, all these experiments done with this type of samples will have the code of PHBC (PHB Cells).

Sample A: 16-10-2013 (PHBC A) 54% PHB
Sample B: 22-10-2013 (PHBC B) 51% PHB
Sample C: 26-10-2013 (PHBC C) 50% PHB
Sample D: 11-12-2013 (PHBC D) 59%PHB

As it has been said before, these samples of PHBC still contained high amounts of water, and according to previous experiments done before with pure PHB and PHBV [15] the properties of PHB and PHBV didn´t change at temperatures below 60°C while with high heating temperatures there was significantly increase in the polymer degradation, so to remove the water temperatures around 60 °C will be used.

To do the experiments with PHBC in the same conditions as previous experiments done with pure PHB [12] the samples of PHB Cells were put in a freeze drier that had a condenser temperature high enough to remove the water and not degrade the rest of the sample.

More than one freeze drying run was necessary to remove all of the water content, therefore the results using PHB Cells in the first and second freeze drying had a starting material which was not completely dry. These experiments were just done to remove the amount of water and then see if the results of MC conversion were similar to the ones obtained with pure PHB. After the second freeze drying the samples could be put in an infrared drier to see the dry percentage and then see if the water was completely removed or it was necessary more than one freeze drying.

The following experiments will ty to answer the question if there is any difference when using pure PHB and PHB Cells when the reaction conditions used are the optimal ones for the reaction with pure PHB done by my supervisor Jurjen Spekreijse: T=200 °C, t= 6h, 0.600g PHB, 10 mL of methanol and P=15bar -20 bar and also completely dry.

The results of the first freeze drying are as follows in Table 2

Table 2. Results of experiment JHO02.

Entry	Sample	MC (%)	CA (%)	3HBMe (%)	Losses (%)
1	PHBC A	53	3	3	41
2	PHBC B	46	2	7	45
3	PHBC C	46	13	16	24
4	PHBC D	45	3	8	43

Reaction conditions: T=200 °C, t= 6h, PHBC (0.600g PHB), 10 mL of methanol, no 3HB (%) was observed. Pressure unknown due to a problem with pressure sensor.

It could be seen looking at the samples that there was still some water present and that the only one that was completely dry was sample D The results obtained are very similar to the ones obtained with pure PHB in Table 1, however the samples were put again in a freeze drier to make sure that all the water was completely removed

After the second and third freeze drying, the samples were put in an infrared drier to know the exact dry matter percentage that was present in the samples. The conditions didn't change from JHO02 (1st FD), the only that changes is that now the presence of water is much lower in samples A and B.

The results obtained after the 2nd and 3^{d} FD can be seen in Tables 3, 4:

Table 3. Results of experiment JHO04 (2nd FD)

Entry	Sample	MC (%)	CA (%)	3HBMe (%)	Losses (%)	Dry (%)
1	PHBC A	49	2	18	31	95
2	PHBC B	57	2	5	36	97
3	PHBC C	52	1	6	41	77
4	PHBC C	50	3	9	38	99

Reaction conditions: T=200 °C, t= 6h, PHBC (0.600g PHB), 10 mL of methanol, no 3HB (%) was observed. Pressure unknown due to some problems with pressure sensor.

As can be seen in Table 3, the conversion for sample A and B are 49% and 57% of MC, there is also some water present in sample C. All of the samples show results quite similar to the ones obtained from pure PHB in Table 1

Table 4. Results from experiment JHO11 (3d FD)

Entry	Sample	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)	Dry (%)
1	PHBC A	30	2	15	54	20	95
2	PHBC A JHO12	35	1	6	58	21	96
3	PHBC C	49	1	7	43	22	96
4	PHBC D	44	2	-	54	19	99

Reaction conditions: T=200 °C, t= 6h, PHBC (0.600g PHB), 10 mL of methanol, no 3HB (%) was observed.

After the third freeze drying, the results in Table 4 show that the behaviour of PHB Cells after different freeze drying runs are comparable. Sample A shows a much lower conversion of MC after the third freeze drying. This experiment was done in duplicate (Table 4, entries 1 and 2) and the conversion was still a bit lower than the rest. In general, in all the samples the conversion of 3HBMe (from 6% to 15%) is higher than the one obtained for pure PHB (3%). The losses observed in the experiment of PHBC for pure PHB were lower from 28% to 35%, than with pure PHB (28% to 35%) which are basically gases like CO or CO^2 and propylene originated from a decarboxylation reaction, reported for CA (crotonic acid) [32].

It can be assumed looking at the results in Figure 5 (see below), that the different samples of PHB Cells have the same behaviour in the reaction to obtain MC once the samples are as dry as the samples used of pure PHB. The composition of all the samples are different because they were taken at different dates, but as we can see in terms of MC conversion the results are not affected by it.

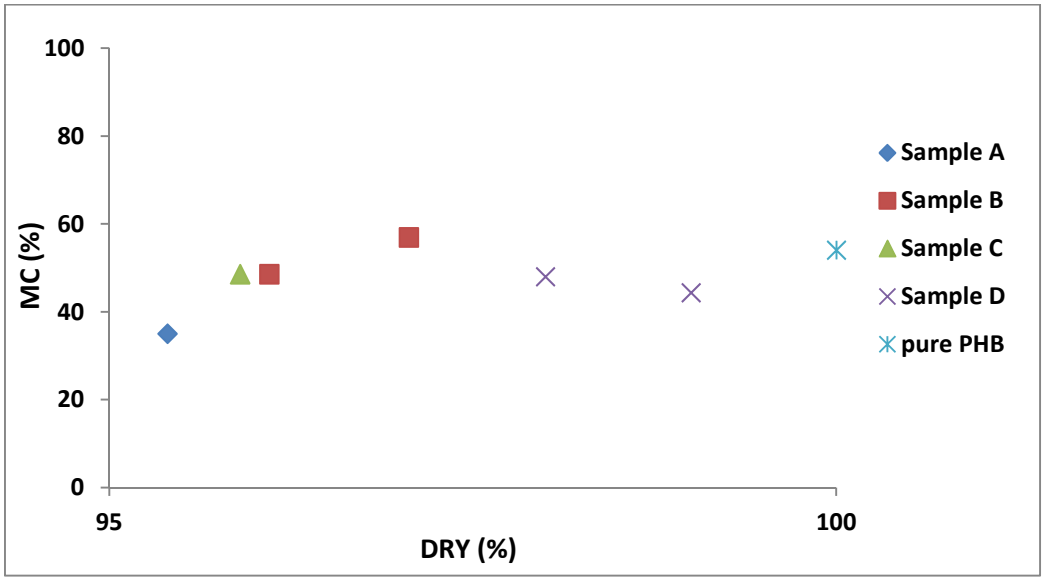


Figure 5. Effect of Freeze drying in MC (%).

As it can be seen in Figure 5, there is not too much difference between the second and third freeze drying, but as it can be seen in later experiments testing different amounts of water, its presence in the samples produces several changes in the conversion of the products. The results obtained are very similar to the ones obtained with pure PHB in the first experiments shown in Table 1.

Effect of temperature in degradation of PHB Cells versus pure PHB

The thermal degradation of PHB using a TGA according to previous experiments [16] showed that degradation of neat PHB and PHBV have revealed it occurs rapidly near the melting point according to mainly a random chain scission.

According to some authors the fermentation residues[17] or plasticizers [18] have influence on the PHB degradation. According to these last researches [16] this fermentation residues formed basically by a mixture of lipids like free fatty acids, mono-, di- and triacylglycerols, phospholipids, cholesterol and cholesterol esters and also ammonium compounds had almost no influence on the polymer main degradation step.

Inspired by these last experiments, a TGA has been done to the samples taken from the fermentation stage (PHB Cells) to see if the residues which are different from the previous experiments because the samples used were taken after depletion of the ammonium with ATU (Allylthiourea), had any effect in the degradation of the PHB.

TGA was also done because in previous reports [6] at the maximum degradation temperature of 310°C it was obtained 63% CA from PHB, but there were not studies about an average degradation temperature and it wasn't very sure if the maximum conversion of CA was obtained at the maximum degradation temperature or if it was higher at a lower average temperature.

Experiments previously done with pure PHB [19] showed that the average degradation was from 150 to 240°C (see Figure 6).

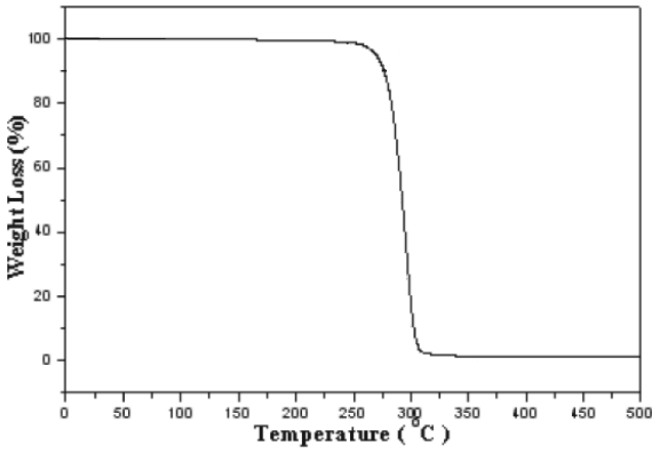


Figure 6. TGA for pure PHB.

As we can see in Figure 6, the onset degradation temperature for the degradation of pure PHB stars at *circa* 250°C and ends at 300°C, where everything has been degraded.

Some experiments with TGA were done to know if there was a difference in degradation temperature between the samples of pure PHB and the samples of PHB Cells obtained from the pilot plant and also in samples of PHBV with 20%Valerate. The samples of PHB Cells were the ones obtained after the second freeze drying.

The samples of PHB Cells show the following behaviour:

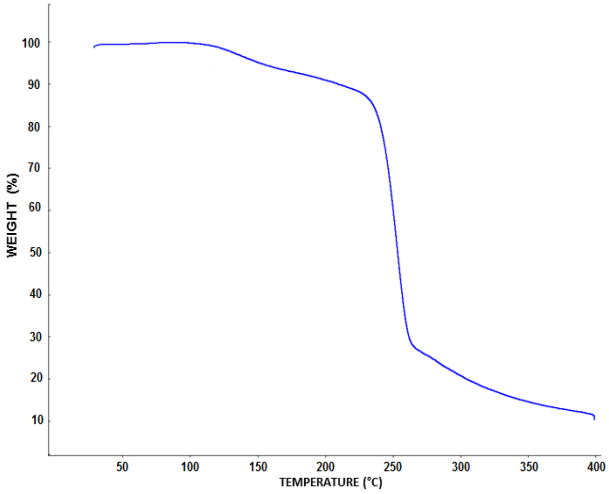


Figure 7. TGA for PHBC D.

The TGA than can be seen in Figure 7 corresponds to sample D, which is the driest sample of PHB Cells. The results are a bit different to the ones obtained for pure PHB in Figure 6. The TGA was also done for another sample of PHB Cells (Sample C), the results can be seen in Figure 8.

According to the results obtained, it can be assumed that the presence of residues from the fermentation doesn´t have any influence on the polymer main degradation step, which confirms the idea shown on [16]. Another TGA was done to another sample of PHB Cells that still have some water, to see the difference with Sample D that was completely dried.

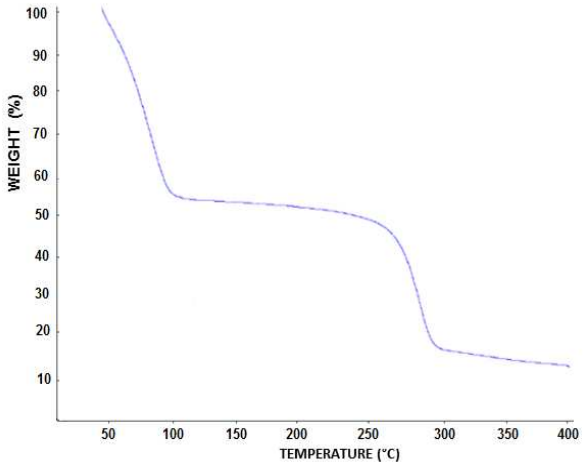


Figure 8. TGA for PHBC A.

In Figure 8, it can be seen that there is first drop of weight (%) when the temperature is around 50°C, which can be due to the presence of water in the sample. According to the TGA, the results obtained before make sense because the amount of water was still significant in the sample, making MC conversions get lower. A TGA was also done to the rest of samples from PHB Cells (B, C) which can be seen in the appendix.

The difference between samples without valerate and with 12% and 19% [20], can be seen in the following figure as follows:

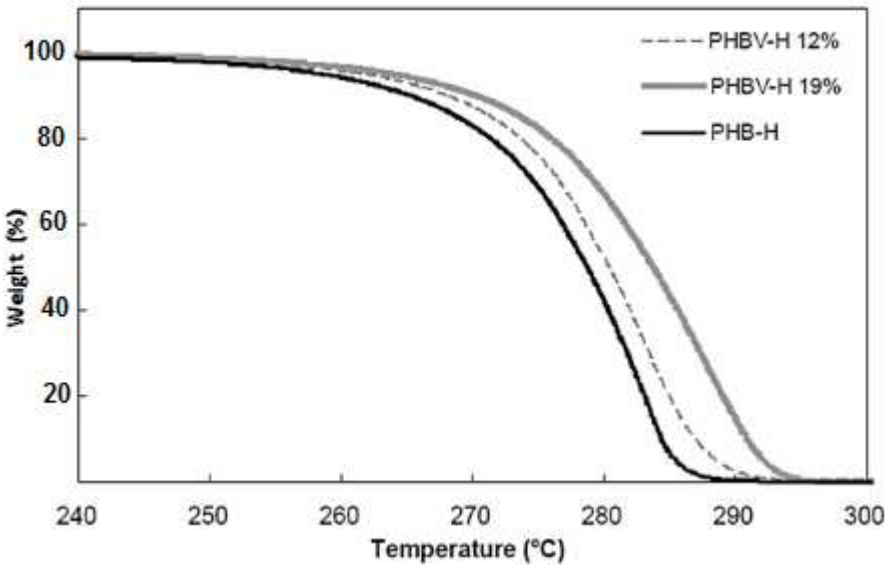


Figure 9. TGA for samples of pure PHB and PHBV (12 %, 19 %).

As it can be seen in Figure 9, the degradation apparently happens at the same temperature, the presence of valerate doesn't make the sample have different degradation as the purified PHB. Higher HV content contributed to higher stability towards the thermal degradation, the thermal stability of polyesters increases with the increasing number of structural carbon units in the polymers [20].

Following the idea of the experiments done by TGA, several experiments have been done at the same time as TGA to see the difference in the degradation of PHB Cells and pure PHB.

According to previous experiments [12], no esterification takes place at temperatures below 150°C and also as it could be seen in the TGAs shown previously in Figures 7, 8 the optimal degradation temperature for the samples of PHB Cells couldn´t be seen through this technique, so the next experiment has been done to see at which temperature the samples from PHB Cells get an optimal conversion and then compare the results obtained with pure PHB and determine if the presence of biomass or other compound which are present in the samples have any effect on the degradation temperature (see appendix, PHA content and substrate (cells Delft University).

Table 5. Results obtained from JHO15 (Different Temperatures) with PHB Cells.

Entry	T (°C)	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
1	180	40	11	21	28	11
2	200	52	2	3	45	17
3	220	40	5	1	54	17

Reaction conditions: t= 6h, PHBC B (0.600g PHB), 10 mL methanol, no 3HB (%) was observed. Average of duplicate experiments.

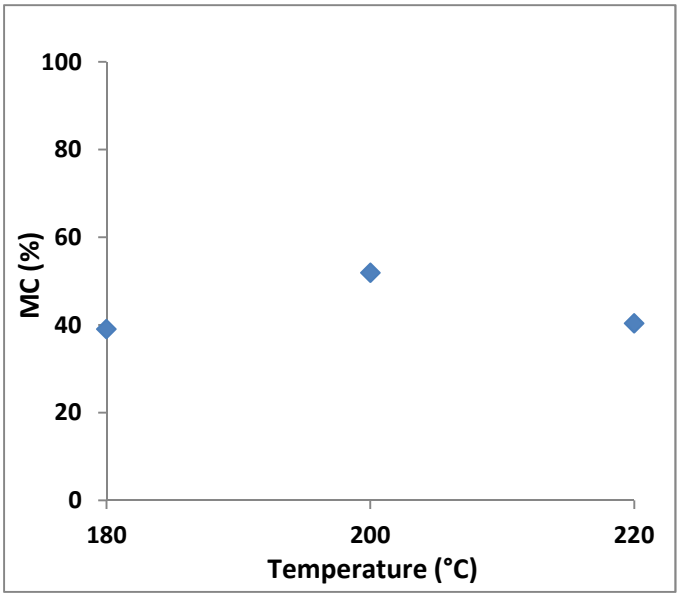
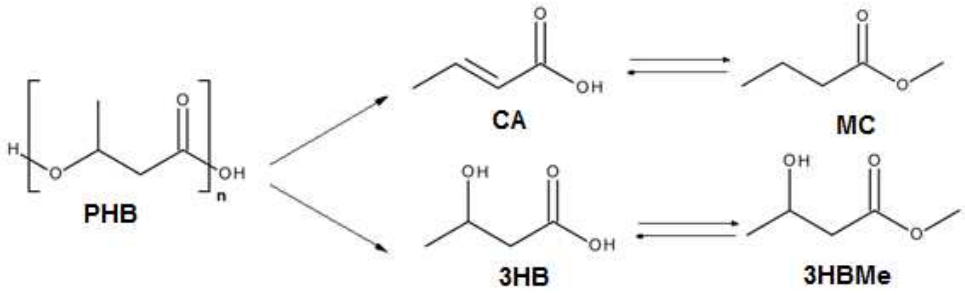


Figure 10. T vs MC (%) in JHO15.

As we can see in Table 5 and Figure 10, the MC conversion in this range of temperature is similar. In entry1, the results are a bit lower when the temperature is 180°C, while it gets higher when the temperature reaches 200°C in entry 2 getting a conversion from 51 to 53%. In the entry 3, at the highest temperature of 220°C, the conversion reaches conversions between 38% and 43%.

It can also be seen that when the temperature is 180°C, the conversion of 3HBMe reaches 21 %, which is higher than with 200°C 3% and 220°C with just 1%. This can be because the reaction hasn't reached the optimal conditions for the production of MC and the CA hasn't fully reacted to form MC, so there is still some left (see Scheme 3).

The increase of 3HBMe could be explained as an effect of the speed in the reaction, at temperatures below 200°C the speed reaction to form 3HBMe is higher than MC formation and when the temperature increases to 200°C the speed of MC production speeds up faster than the trans-esterification reaction because the conditions are more in favour for the formation of MC.



Scheme 3. Reaction of PHB in presence of MeOH [10].

As it can be seen in Table 5, the losses increase during the experiments when the temperature also increases. This losses are mainly gases like CO, CO_2 and also propylene and ethylene according to a GC-MS analysis done by my supervisor [12].

After analyzing the TGA done on the samples of PHB Cells and after studying the results obtained in the previous experiment with different temperatures we can confirm the hypothesis of decarboxylation of products shown in the introduction involving pure PHB, therefore the behaviour of pure PHB and PHB Cells in terms of degradation follows the same pattern.

As it can be seen in Figure 8 the TGA is different when there is still water present in the samples from PHB Cells. The next step will be to know if the presence of water affects the MC conversion in the same way as with pure PHB.

Impact of presence of water on MC and 3HBMe conversions.

In recent researches that are being done in Eindhoven University [1] studying the whole process from PHB to MC and now it is also important more research is being done to know the PHB conversion in water presence.

It is shown in some studies [21] that the degradation of PHB occurs through a random chain scission in the non-aqueous conditions while the degradation of PHB in the presence of water occurs through surface hydrolysis with no change of molecular weight.

According to [22] when the morphology of the PHB has holes on the surface, it allows water molecules to come into contact with the surface and the polymer around the holes starts to degraded. As the holes become bigger and bigger more bacteria and water molecules can fill the big holes, leading to further degradation. On a smooth process surface, this process would be much more difficult.

As the samples from pure PHB used in previous experiments have been already process through a purification step, the surface of these samples is thought to be smooth and more crystalline, while samples from PHB Cells as they haven´t been processed in any treatment, its morphology is not well known.

Inspired by these researches that showed that depending on the morphology of the process the degradation of the PHB could be higher, some experiments were done with samples taken from fermentation (PHB Cells) to see if they showed the same behaviour as previous experiments done with pure PHB [10]

The results are as follows in Table 6:

Table 6. Results of experiment JHO03.

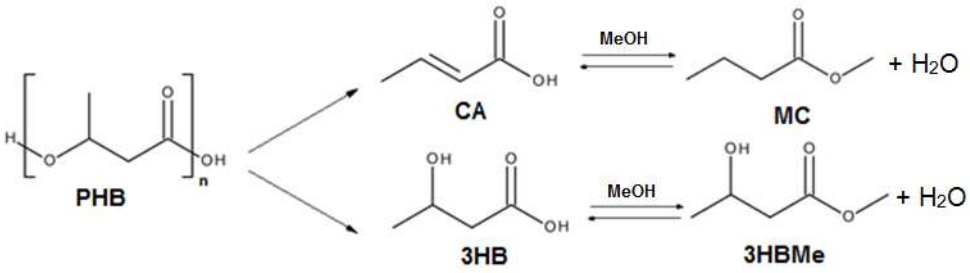
Entry	Sample	MC (%)	CA (%)	3HBMe (%)	Losses (%)
1	PHBC A	5	24	16	13
2	PHBC B	22	10	5	34
3	PHBC D	30	2	-	60
4	PHBV20	3	30	20	49

Reaction conditions: T=200 °C, t= 6h, PHBC (0.600g PHB), 10 mL of methanol. All experiments with 10 mL water, no 3HB (%) was observed.

Although the samples had still some water in them before adding even more water to test its effect, it's sure to say that the presence of water makes the conversion of CA and 3HBMe go

higher and the one of MC go lower. The experiment with PHBV20 also shows that with water there is a clear decrease in MC conversion and a high increase of 3HBMe.

This can be due to the equilibrium of the reaction that can be seen as follows in the Scheme 4.



Scheme 4. Reaction of PHB in the presence of water.

As it can be seen the formation of MC comes directly from the reaction CA has with methanol, the same happens with the formation of 3HBMe which comes from 3HB. The equilibrium that takes place in both reactions is shown in the following equation:

$$K_1 = \frac{[MC][H_2O]}{[CA][MeOH]} \quad K_2 = \frac{[3HBMe][H_2O]}{[3HB][MeOH]}$$

Equation 1. Equilibrium of reactions shown in scheme 2.

In order to get the same value of the equilibrium constant, in the first reaction of CA, when the amount of water increases and the amount of methanol remains constant, the concentration of MC has to decrease and CA has to go higher so the equilibrium doesn't change. The same happens with the second reaction that involves 3HB.

In the following experiment, the reactions were done with PHB Cells (Sample C) with different amounts of water and then a comparative was done with some experiments done by my supervisor Jurjen Spekreijse with pure PHB and see if there is any difference.

Table 7. Results of PHB Cells with different amounts of water.

Entry	Sample	Water (mL)	MC (%)	CA (%)	3HB (%)	3HBMe (%)	Losses (%)	P (bar)
1	PHBC C (Table 4)	-	49	1	-	7	43	22
2	PHBC C	2	41	7	8	31	12	20
3	PHBC C	4	21	12	10	31	26	21
4	PHBC C	6	15	19	15	39	12	21
5	PHBC C	8	14	23	17	22	24	20
6	PHBC C	10	12	25	9	42	12	21
7	PHBV20	10	3	30	20	49	-	21

Reaction conditions: T=200 °C, t= 6h, 10 mL of methanol, PHBC (0.600g PHB) and PHBV20 (0.600g PHB). Average of duplicate experiments (JHO08 and JHO21).

Table 8. Results of pure PHB with different amounts of water. Results from my supervisor Jurjen Spekreijse.

Entry	Water (mL)	MC (%)	CA (%)	3HB (%)	3HBMe (%)	Losses (%)	P (bar)
1	-	54	15	-	10	21	20
2	2	50	13	-	-	37	21
3	3	29	27	13	8	23	19
4	4	22	34	15	4	25	19
5	4.7	11	20	13	3	53	19
6	8	13	25	24	4	34	19

Reaction conditions: T=200 °C, t= 6h, 10 mL of methanol, 0.600g pure PHB. Average of duplicate experiments (JHO08 and JHO21).

The results obtained in Tables 7 and 8 for PHB Cells and pure PHB, are shown in Figure 11 as follows:

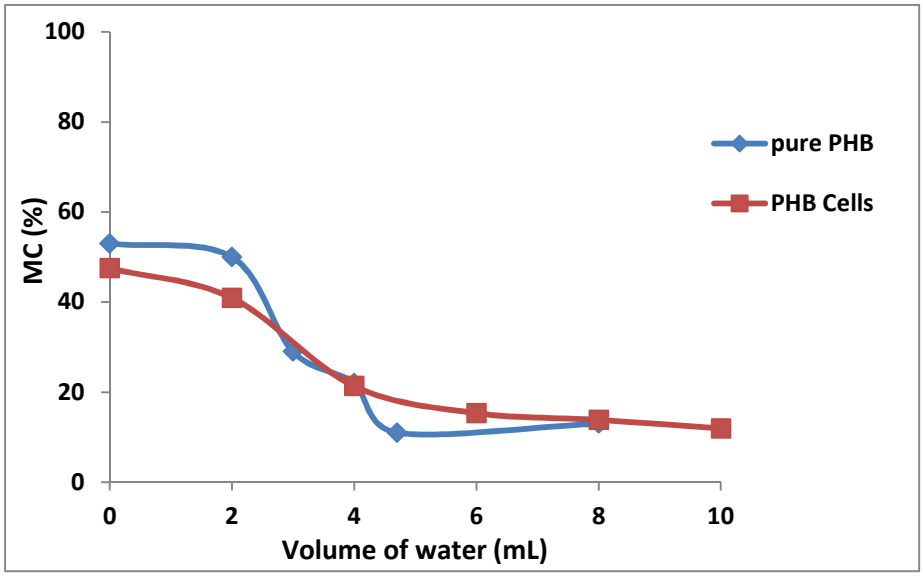


Figure 11. Difference of MC(%) with and without water.

As it can be seen in Figure 11, the effect of water is very similar in both samples. As the amount of water increases the MC conversion decreases and then because of the equilibrium Ca increases its conversion. The reaction of 3HB (Scheme 3) also increases its speed and the 3HB but because of the presence of water, 3HBMe conversion increases from 7% without any water (Table 8) to 42% with 10 mL water.

Comparing Tables 7 and 8, it is shown that when the amount of water increases, with pure PHB the decrease of MC turns into an increase of losses like gases CO, CO_2 and also CA, while the samples that come from PHB Cells and PHBV20 (20% Valerate), the conversion of 3HBMe increases drastically, as it can be seen in [1], the percentage of valerate present in the samples that come from PHB Cells is 16%.

The valerate together with the presence of water makes the 3HBMe conversion go up, the properties of pure PHB and valerate [23] Are shown in Table 9 as follows:

Table 9. Melting T and crystallinity of PHB and PHBV20.

Properties	PHB	PHBV20
Melting T (°C)	180	173
Cristallinity (%)	72	51

The valerate content of the PHBV copolymers act as plasticizer producing a small, but noticeable change in the overall rate of degradation. It seems that another factor that favored the slightly faster degradation of PHBV than PHB is its lower crystallinity (Table 9) as the larger amorphous regions enhanced the permeation of water molecules and the subsequent hydrolysis of ester bonds [23].

The increase in the degradation rate at higher pH values probably indicates that the degradation of the polymer is mediated by hydroxyl ion, under aqueous condition, the mechanism is thought to occur through ester hydrolysis [24].

In the following experiment it is also studied the effect of different amounts of water, but this time the amount of pure PHB during the experiment was also changed from 0.600g to other amounts as follows in Table 10:

Table 10. Results from experiment with different amounts of pure PHB and water (JHO10).

Entry	Mass (g)	Water (mL)	MC (%)	CA (%)	3HB (%)	3HBMe (%)	Losses (%)	P (bar)
1	1.8	2	39	22	10	6	24	20
2	1.8	4	23	46	3	1	27	16
3	1.8	7	17	29	10	3	42	22
4	0.3	2	17	33	-	3	47	23
5	0.3	4	17	32	14	-	37	22
6	0.3	7	9	37	24	4	26	19

Reaction conditions: T=200 °C, t= 6h and 10 mL of methanol.

As it can be seen in Table 10, the MC conversion is clearly lower than the previous experiment with just 0.600g of PHB Cells. The same amount of water with different amounts of pure PHB doesn't show a very different behaviour unless when the presence of water is (2mL) that the results are better when the amount of PHB is 0.600g than when it's 1.800g, so in general is more important that the amount of water than the amount of pure PHB used in the experiment,

The effect of the amount of water used in the reactions is also analyzed in Table 11 taking into account the results obtained from Tables 7, 8 (results from Jurjen Spekreijse) and 10. The study of the ratios of water/PHB is shown in the following table as follows:

Table 11. Results from pure PHB and PHB Cells to study H_2O /PHB ratio.

Sample	Entry	V_{water} (mL)	MC (%)	water/PHB	m PHB (g)
Pure PHB	1	2	39	1.1	1.8
Pure PHB	2	4	23	2.2	1.8
Pure PHB	3	2	50	3.3	0.6
Pure PHB	4	7	17	3.9	1.8
Pure PHB	5	4	24	6.7	0.6
Pure PHB	6	5	14	8.3	0.6
Pure PHB	7	10	8	16.7	0.6
Pure PHB	8	7	9	23.3	0.6
PHBC C	9	2	41	3.3	0.6
PHBC C	10	4	13	6.7	0.6
PHBC C	11	6	14	10.0	0.6
PHBC C	12	8	16	13.3	0.6
PHBC C	13	10	12	16.7	0.6

The entries 3, 4 in Table 11 have a water/PHB ratio similar and a MC conversion very different. As explained before, the amount of PHB and water is very different and then water seems to be a more important factor than the amount of PHB.

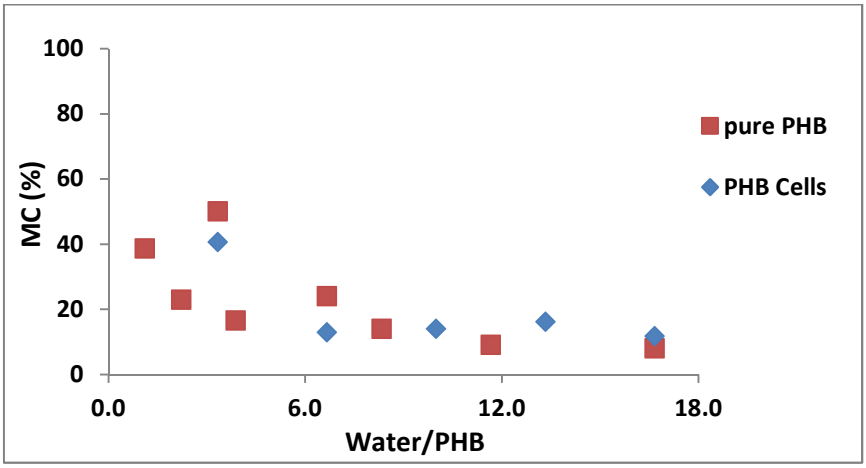


Figure 12. Effect of water/PHB with pure PHB and PHB Cells in MC (%).

As we can see in Figure 12, comparing results from pure PHB and also PHB Cells, the effect of water/PHB is very similar so we can assume there is no difference between pure PHB and PHB Cells.

Reaction development of PHB Cells versus pure PHB

Once it has been seen that the presence of water affects MC conversion the next variable that will be studied is the residence time, which until now it has been settled at 6h (the same as optimal conditions for pure PHB samples). Next experiments will focus on the study of the reaction at different times to see if there is any difference.

The main objective will be to see if the reaction reaches the optimal conditions as with pure PHB at the same time or if because of the presence of some compounds in the samples of PHB Cells the reaction goes faster or slower. It will also be important to see the conversion of the rest of the products. The results obtained at a residence time of 6h were similar between

samples from PHB Cells and pure PHB, but it is not known if the conversion is reached faster with pure PHB or with PHB Cells.

As it can be seen in previous experiments done at different temperatures, the presence of residues from the fermentation were proved to not make the degradation run under lower temperatures. The presence of these residues also come from the nutrient stock and according to [25] there are some metallic compounds that could make the degradation of PHB faster than with just purified PHB.

The variable time was studied to see the effect it has on the conversion of PHB to MC and also in the rest of the components. The experiment was done with different residence times from 1 to 8h. The results obtained are shown in Table 12.

Table 12. Results of PHB Cells using different residence times from JHO06, JHO12.

Entry	t (h)	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
1	1	30	23	23	22	14
2	2	40	10	13	36	16
3	3	46	3	6	46	15
4	4	48	4	13	36	19
5	5	46	6	6	38	21
6	6	48	2	16	49	21
7	7	48	1	4	43	23
8	8	47	1	3	49	20

Reaction conditions: T=200°C , 10 mL methanol, PHB Cells (0.600g PHB), no 3HB(%) was observed. Average of duplicate experiments JHO06 (PHBC D) ,JHO12 (PHBC B).

The results obtained in Table 12 are represented in Figure 13.

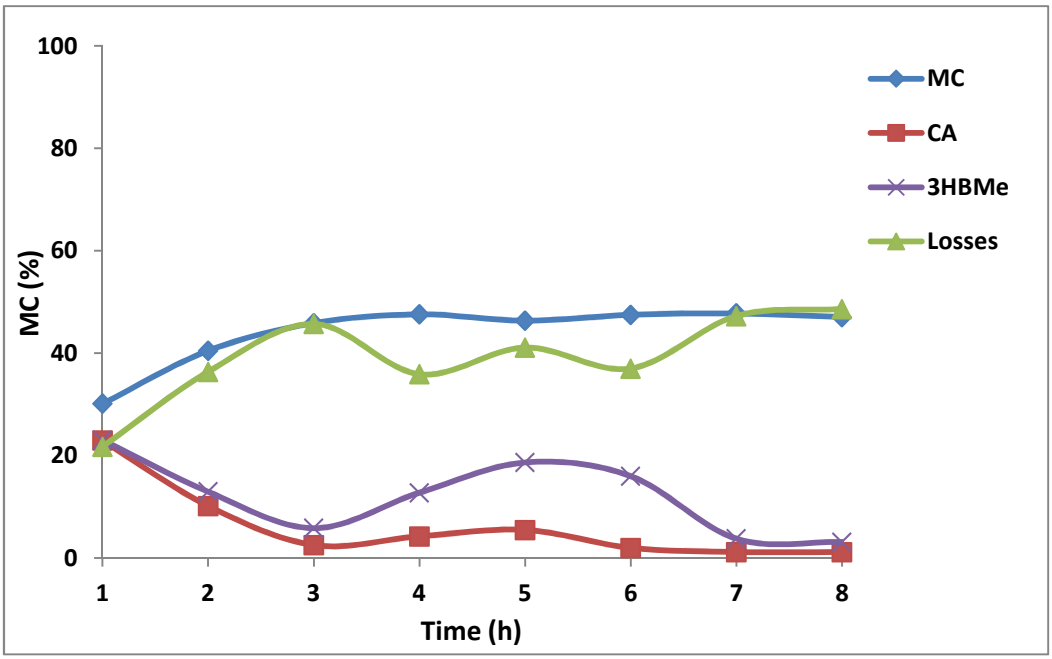


Figure 13. Evolution of MC (%) versus residence time in PHB Cells.

As it can be seen in Table 12 and Figure 13, MC conversion reaches a maximum from 46% to 48% when the residence time reaches 3 hours, then it can be seen that all PHB has reacted and there is no more PHB in the reactor because the MC conversion stays constant.

The rest of the components conversion like CA and 3HBMe go down as the reaction takes place showing a high decrease of the components when the MC reaches the three hours and then stay constant. The differences that can be seen in Table 12 and Figure 13 for 3HBMe and losses from 4h to 6h can be because of the UV response of 3HBMe is small, which induces the error.

The same experiments has also been done using pure PHB (results from my supervisor Jurjen Spekreijse). The result are as follows:

Table 13. Results pure PHB different residence times. Results provided by Jurjen Spekreijse.

Entry	t (h)	MC (%)	CA (%)	3HBMe (%)	Losses (%)
1	1	22	38	2	60
2	2	32	31	1	62
3	3	37	24	1	61
4	4	41	21	1	62
5	5	43	19	1	62
6	6	48	15	0	63
7	8	44	11	1	56

Reaction conditions: T=200 °C, 10 mL methanol, 0.600g pure PHB, no 3HB (%) was observed, P=9 bar. Average of duplicate experiments

The results obtained in the experiments done with pure PHB and PHB Cells in Tables 12, 13 are collected in the Figure 14:

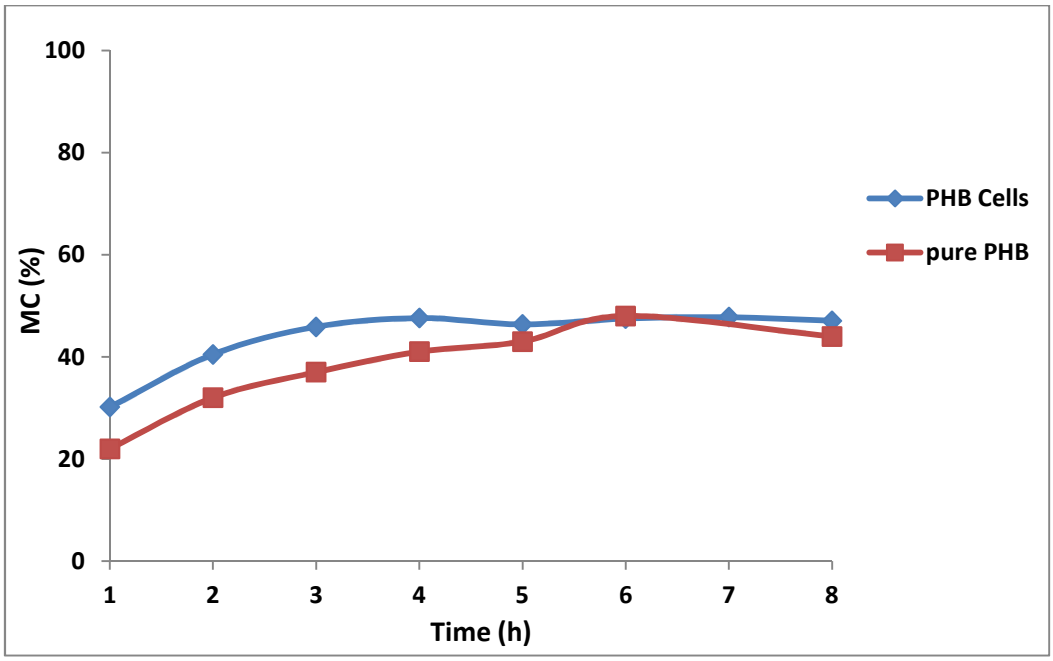


Figure 14. MC (%) vs time (h) in PHB Cells and pure PHB.

As it can be seen in Figure 14, the behaviour PHB Cells and pure PHB is similar, although there is a difference in the residence time required to get a maximum conversion.

The reaction with PHB Cells works faster than with pure PHB, optimal MC conversion for PHB Cells is reached at 3h with 46% while for pure PHB is reached at 6h with 48%. A

possible explanation can be that maybe there are some components in the samples of PHB Cells that act like a catalyst.

- **Study of catalytic effect of $Mg(OH)_2$ on MC conversion**

As the samples come directly from the fermentation process to obtain the PHB, there are some substances that are present in the samples. According to the data given by Delft University, during the fermentation process the nutrient stock solution is shown in Table 14.

Table 14. Components of nutrient stock solution for fermentation process of PHB.

Components	Concentration (M)
NH_4Cl	0.19
KH_2PO_4	0.09
$MgSO_4 \cdot 7H_2O$	0.02
KCl	0.03
Trace elements [a]	

Total volume =1.35L. The samples used for PHB Cells were taken after depletion of the ammonium. There is no NH_4^+ in the samples. [a] Trace elements shown in Table 15.

Table 15. Components of trace elements present in the nutrient stock.

Components	Concentration (M)
EDTA Titriplex III	9.51E-03
$ZnSO_4 \cdot 7H_2O$	4.25E-03
$CoCl_2 \cdot 6H_2O$	3.74E-04
$MnCl_2 \cdot 4H_2O$	1.43E-03
$CuSO_4 \cdot 5H_2O$	3.34E-04
$FeSO_4 \cdot 7H_2O$	6.00E-04
$(NH_4)_6Mo_7O24 \cdot 4H_2O$	4.95E-05
$CaCl_2 \cdot H_2O$	3.14E-03

As it can be seen in Table 15, the main components of the nutrient stock solution are Mg^{2+}, K^+ ($NH_4^+$is not present in the samples because samples were taken after depletion of the ammonium with ATU(Allylthiourea)) and also anions of sulphate. As it has been reported by Gizela Malkova from Eindhoven University, for the degradation reaction of pure PHB with biomass, it has been used p-Toluene sulfonic acid as catalyst, which is a common transesterification catalyst. Unlike the reaction done in by Gizela Malkova, the reaction in our study is done in presence of methanol and the acid can react with it, so instead using an acid another catalyst will be used based in the components shown in the stock solution in Table 14.

The presence of metallic components [20], caused a decrease in the degradation temperature in the experiments done with samples of PHBV (19% Valerate) in the presence of $Mg(OH)_2$ and also facilitated the formation of a double bond by elimination of B-hydrogen which means an enhancement in the conversion of the biopolymer. Previous experiments [26] have shown that with the use of $Mg(OH)_2$ there is a nearly complete selectivity (~100%) to trans-crotonic acid. It is also suggested that Mg catalyst promote the totality of the β-elimination reactions by acting throughout the beginning and main processes, resulting in a lowering in the degradation temperature and the completely selective transformation of PHB.

The results can be seen in Figure 15:

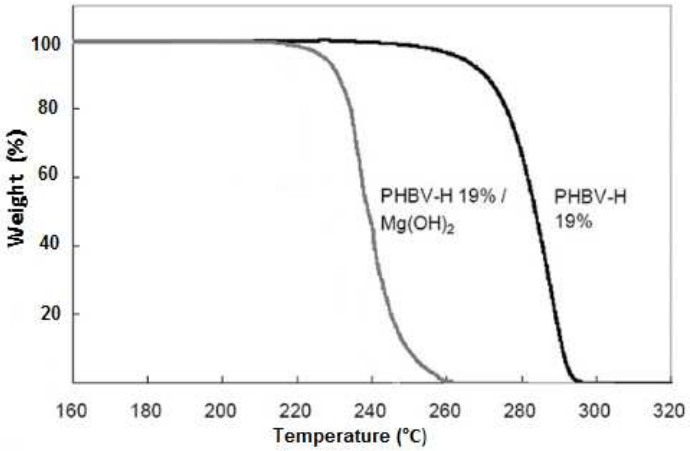


Figure 15. TGA for samples with and without Mg $(OH)_2$.

As it can be seen in Figure 15, the degradation temperature is clearly lower in samples with presence of $Mg(OH)_2$. The difference between samples of pure PHB and PHBV19 could be seen before in figure 9 where it could be seen that there was not too much difference

Magnesium catalyst in form of $Mg(OH)_2$ is a heterogeneous catalyst, which can improve the synthesis methods by eliminating the additional costs associated with conventionally used homogeneous catalysts. Heterogeneous catalysts are economically and ecologically important when compared with homogenous catalysts because they are environmentally benign, much easier to separate from liquid products, they facilitate the purification stages, can be reused, are non-corrosive, have high thermal stability and present fewer disposal problems [27]

It is believed that conversion of PHB into crotonic acid can be increased considerably if the thermal degradation proceeded with the aid of catalysts of magnesium hydroxide, which are also not harmful and do not cause any side reaction with the polymer, which is also an important factor for the chemical recycling process. The use of magnesium salt as catalyst was able to reduce the activation energy of degradation process and consequently reduce the formation of dimer and trimer [20].

It has been seen [28] that the presence of metallic Lewis acid sites in the silicate layers enhances the thermal degradation of PHB by catalyzing the hydrolysis of ester linkages.

According to the theory of Lewis acidity, hydroxyde groups can act like Lewis bases, because they can donate a lone pair of electrons and cations like Mg^{2+}, which are invariably complexed with additional ligands, are often sources of coordinatively unsatured derivatives that form Lewis adducts upon reaction with a Lewis base. The stability of the intermediate increase due to the effect of the hydroxide groups shown in the first part of Figure 16 and also it can be because of the cation Mg^{2+} in the following part.

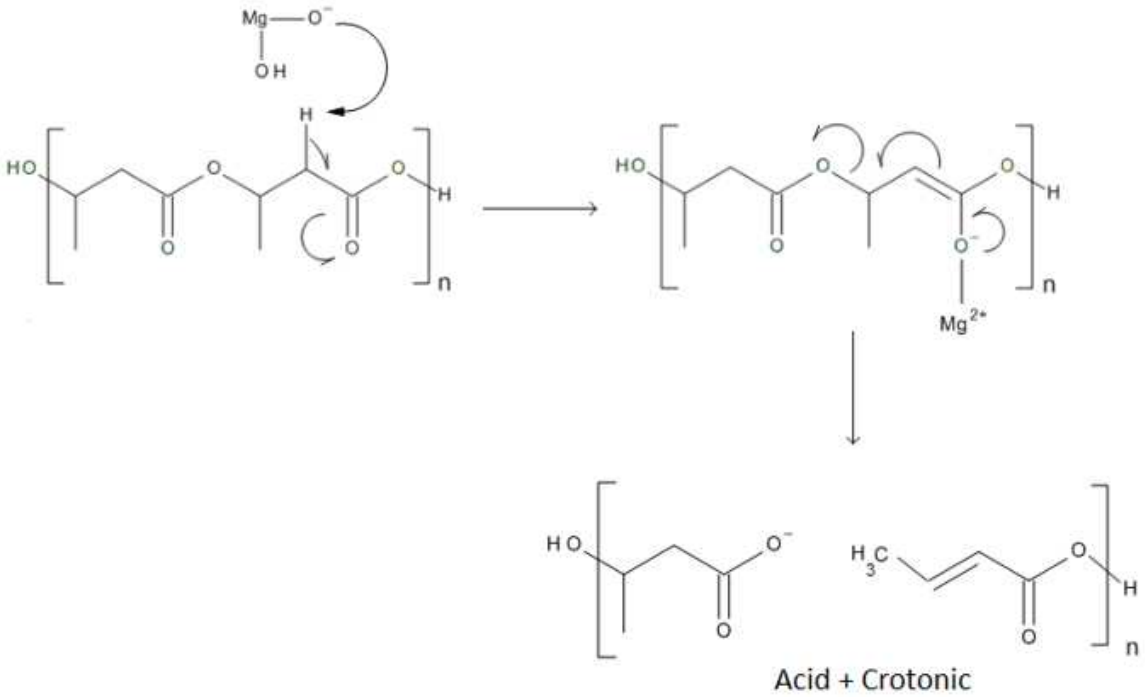


Figure 16. Potential mechanism (E1cB-mechanism) with Mg $(OH)_2$.

The following experiment will focus on the use of $Mg(OH)_2$ with pure PHB to see if its presence makes the reaction goes faster than the one shown in previous Figure 10 for the same samples and see if the results look similar as the ones obtained with PHB Cells during the experiment with different residence times in Figure 16.
The results are shown in Table 16:

Table 16. Results of MC conversion pure PHB with catalyst.

Entry	t (h)	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
1	1	42	28	4	26	17
2	2	53	16	5	28	17
3	3	60	5	2	34	18
4	6	58	6	2	34	17

Reaction conditions: T=200°C , 40 mg $Mg(OH)_2$ as catalyst (10%mol), 10 mL methanol, 0.600g pure PHB, no 3HB(%) was observed. Average of duplicate experiments.

The results obtained from Table 16, compared to the experiments done with pure PHB and PHB Cells are shown in the following Figure 17:

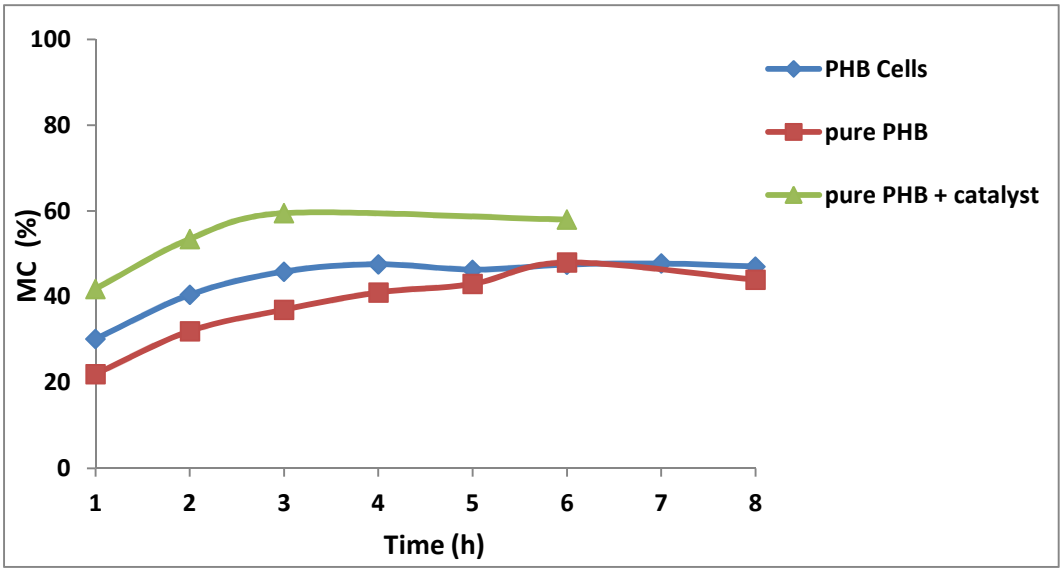


Figure 17. MC (%) vs time in pure PHB with and without catalyst and PHB Cells. T=200°C, 10 mL methanol.

As it can be seen in Figure 17, the results obtained with pure PHB with $Mg(OH)_2$ as catalyst show a higher conversion than experiments without catalyst. Similar to PHB Cells, the reaction seems to reach optimal conversion of MC at a residence time of 3h in a range from 58% to 60% instead of 6h that take for the reaction with only pure PHB, so we conclude that the presence of Mg acts like a catalyst. The results are higher than the results with PHB Cells that have already salts in their composition in form of $MgSO_4.7H_2O$, which can be due to the fact that the PHB Cells used for the experiment have a 2.2% molar ratio of $Mg(SO_4).7H_2O$ compared to PHB, while in the samples of pure PHB with $Mg(OH)_2$ the concentration is of 10% molar ratio compared to pure PHB.

These experiments have shown that the reaction with PHB Cells is faster than the reaction with pure PHB due to the presence of some nutrients that were used in the nutrient stock solution to obtain the PHB, in this case tested with the presence of the cation of Mg^{2+}.

Use of different amounts of solvent

For now all the experiments have been done with the same amount of solvent (methanol) as previous experiments done with pure PHB [12]. According to the studies shown in [29], the formation of methyl esters from vegetable oils involves the use of methanol to do the transesterification step. These experiments showed that the methyl ester contents can be achieved rapidly in one pass, while in those cases where extra methanol and cosolvent are used, there are costs associated with extra distillation. It can also be seen for the production of methyl esters from soybean oil [30], that with the increasing of methanol-soybean molar ratio, since transesterification is reversible in nature, there can be a reverse reaction between glycerol and methyl esters making the glycerol dissolve in methanol and leading to a difficult separation between the methyl ester and glycerol.

In these experiments the methanol present in the reaction turns to gas increasing the pressure inside the reactor. Depending on the pressure used during the reaction, the methanol will react

either with the CA or the PHB present in the bottom in the reactor, so the amount of methanol used during the reaction can be an important factor during the reaction.

The following experiment tests different amounts of methanol in samples from PHB Cells and from PHBV20.

Table 17. Results of experiment JHO07.

Entry	Sample	V MeOH (mL)	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
1	PHBC D	20	36	5	20	39	30
2	PHBC D	15	42	4	6	48	17
3	PHBC D	5	47	1	1	51	16
4	0.6 g PHBV20	10	60	5	14	21	23
5	0.6 g PHBV20	15	32	19	15	34	27
6	1.2 g PHBV20	10	64	5	6	25	20
7	2.4 g PHBV20	10	59	22	6	13	17

Reaction conditions: T=200 °C, t= 6h, PHBC (0.600g PHB), no 3HB (%) was observed.

As it can be seen in Table 17, the results in entries 1, 2 show that the conversion of MC goes clearly down when the amount of methanol is higher than 10 mL from 36% to 42%. The amount of methanol also affects the average pressure of the reaction because under these reaction conditions the methanol is in gaseous form, which increases the pressure. The conversion of 3HBMe increases to reach the equilibrium. Entry 3 is the same experiments as entries 1, 2 but this time with just 5 mL of methanol and the MC conversion (47%) has a similar value as experiments shown in Table 4 in experiments with PHBC that reaches from 44% to 49% done with PHB Cells and 10 mL of methanol.

The experiments done with PHBV20 show in Table 17 have a conversion of MC also is significantly lower compared with PHBV20 in standard conditions (entry 4) when the amount of methanol used is higher than 10 mL (entry 5).

Due to technical issues the amount of methanol couldn't be decreased from 10 mL because the reaction cannot take place as it does not get enough height in the reactor to reach the temperature probe, so the amount of PHB was increased keeping constant the methanol at 10 mL, entries 6,7 show that a MC conversion similar to the one obtained in entry 5, although the pressure is quite different, it shows a similar pattern as with PHB Cells when the MC conversion is higher when the amount of methanol is lower than 10 mL

The following experiment tests different amount of methanol in PHB Cells from 4 to 9 mL of methanol. The results are as follows in Table 18:

Table 18. Results from experiment JHO13. PHB Cells. Different amounts of methanol.

Entry	V MeOH (mL)	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
1	4	42	4	4	50	12
2	5	45	3	3	50	15
3	6	43	1	-	56	17
4	7	50	1	-	48	16
5	8	59	2	4	35	18
6	9	53	1	5	41	19

Reaction conditions: T=200 °C, t= 6h, PHBC B (0.600g PHB), no 3HB (%) was observed.

As it can be seen in Table 18, MC conversion was higher when the amount of methanol used was only 5mL than with amounts higher than 10mL, the results obtained from Table 18 also show higher MC conversions.

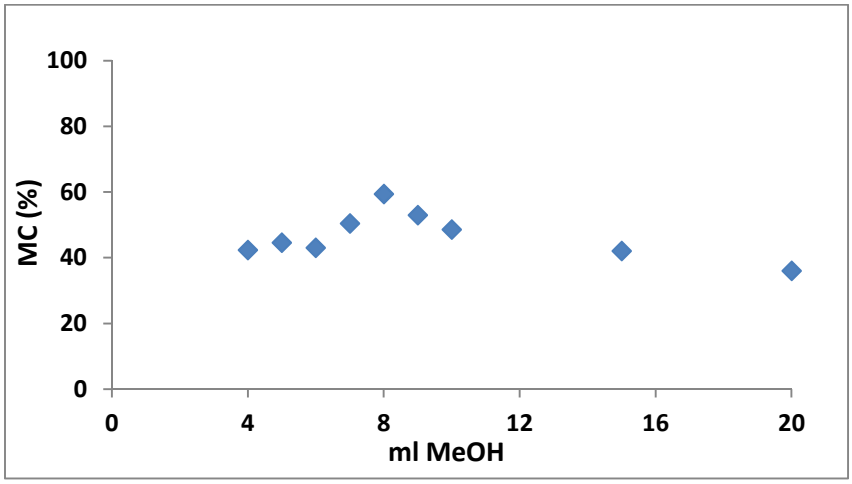


Figure 18. Results from JHO07 and JHO13 to study effect of methanol vs MC(%). T=200°C, t=6h, PHB Cells and PHBV20 (0.600g PHB).

As it can be seen in Figure 18, when the samples come from PHB Cells, MC conversion keeps constant at 40% when the amount of methanol increases to 10 mL and then it goes slightly down, getting a small peak when the amount of solvent used goes from 7mL to 9mL of methanol.

The same experiment as in Table 18 was done with PHBV20 with amounts of solvent from 5mL to 9mL, to see if the higher presence of valerate had any effect in MC conversion. The result are as follows:

Table 19. Results from experiments JHO17. PHBV20. Different amounts of methanol.

Entry	V MeOH (Ml)	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
1	5	45	11	2	42	12
2	7	59	10	4	27	14
3	9	65	7	2	26	14

Reaction conditions: T=200 °C, t= 6h, PHBV20 (0.600g PHB), no 3HB (%) was observed. Average of duplicate experiments.

The difference between PHBC B (Table 18) and PHBV20 can be seen in the following Figure:

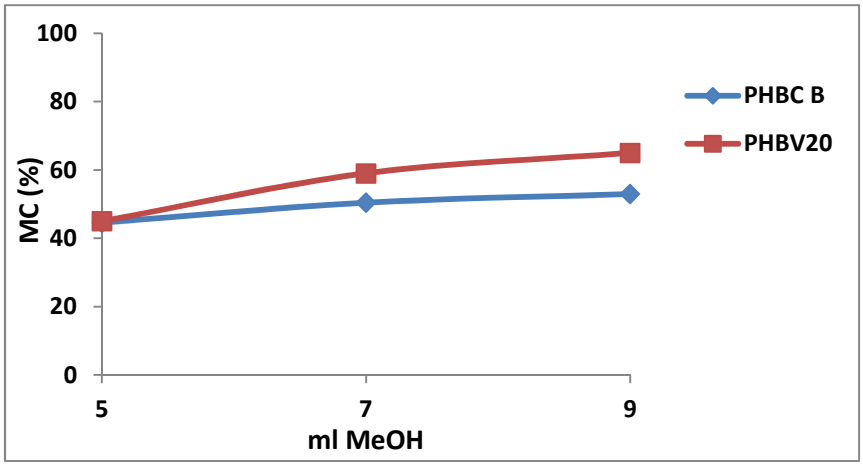


Figure 19. Difference between PHBC B and PHBV20

The results shown in Figure 19 show similar conversion of MC when the amount of methanol is below 10 mL. When the amount of methanol is 5 mL the results are the same with 45% conversion and when is 9mL the results are also similar with PHBV20 65% and PHBC B 53%.

Behaviour of PHB Cells versus pure PHB and catalytic effect of $Mg(OH)_2$ at different pressures

The pressure used until now has been settled between 15-20 bar according to the experiments done by my supervisor Jurjen Spekreijse where the conversion was the optimal one, reaching a MC conversion of 54% [13].

The behaviour of the reaction with pure PHB and PHB Cells at different pressures can be different because methanol can maybe react in a different way because of the presence of valerate, previously mentioned in the experiments done with water.

When the reaction starts, the PHB turns to CA because of the high temperatures and then reacts with methanol forming MC. According to the data obtained from [31] the vapor pressure of methanol is 40 bar when the temperature is 200°C.

At the beginning of the reaction the bottom of the reactor where there is PHB with methanol is heated by the cooling system, making the methanol turn from liquid to gas phase. When the methanol turns to gas, it goes to the top of the reactor where the temperature is lower, it condenses again into liquid and then goes back to the bottom of the reactor. Depending on the pressure used during the reaction, the condensation of methanol takes place near the bottom of the reactor of near the top of the reactor, reacting with PHB or CA respectively as it can be seen in figure 20:

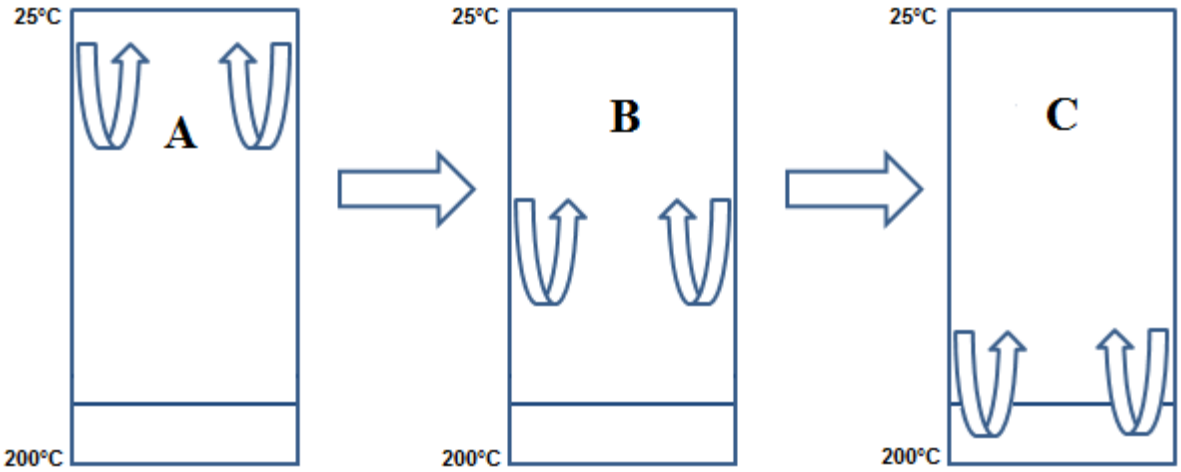


Figure 20. Behaviour of methanol when pressure increases inside the reactors. P(A)<P(B)<P(C)

Picture A shows the behaviour of the reaction when the pressure used is below 10 bar where the methanol turns from liquid to gas before reaching the CA and goes to the top of the reactor before reaching the bottom, MC conversion goes down and losses increase because of the degradation of PHB, picture B for pressures from 15 bar to 20 bar where the methanol is in contact with CA it reaches optimal conversions of MC and picture C for pressures above 30 bar the methanol reacts with the PHB that is present in the bottom of the reactor doing the transesterification and increasing the conversion of 3HBMe.

Keeping constant the amount of methanol used and changing the pressure can also affect the reaction as it has been tested in previous experiments with pure PHB [12]. When the pressure used for the experiment overpassed 27 bar, methanol instead of reacting with CA reacts with either the PHB present in the bottom of the reactor or it just degrades forming CO and CO_2 which are products from the decarboxylation of PHB. The results obtained for pure PHB at different pressures can be seen in Table 20 as follows:

Table 20. Results from pure PHB with 10 mL methanol at different pressures. Results provided by my supervisor Jurjen Spekreijse.

Entry	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P bar)
1	16	36	0	48	3
2	40	12	1	47	8
3	45	15	0	40	9
4	48	14	2	36	10
5	37	16	3	44	10
6	56	9	8	27	15
7	56	11	4	29	15
8	61	6	8	25	17
9	60	9	2.1	29	19
10	53	7	12	28	21
11	45	5	16	34	25
12	42	8	17	33	28
13	41	5	20	34	29
14	38	9	26	27	32
15	17	10	17	56	36
16	18	10	17	55	39
17	17	9	18	56	42
18	13	8	17	62	42

Reaction conditions: T=200°C, t=6h, 0.600g pure PHB, 10 mL methanol, no 3HB (%) was observed.

In the next experiment the amount of solvent used during the reaction was changed from 10mL to 8mL. The aim of this experiment will be to compare the results obtained at different pressures with the previous experiments at 10 mL.

Table 21. Results from with 8mL methanol at different pressures with PHB Cells.

Entry	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P bar)
1	42	11	5	42	6
2	46	6	5	43	10
3	52	2	6	39	14
4	47	1	-	52	15
5	42	2	-	56	17
6	50	1	6	43	19
7	54	3	-	44	20
8	57	3	10	30	22
9	39	5	44	13	28
10	37	9	50	3	37
11	36	10	58	-	37

Reaction conditions: T=200°C, t=6h, PHB Cells (0.600g PHB), 8 mL MeOH, no 3HB (%) was observed. Results from JHO16 (PHBC B) and JHO19 (PHBC C).

The results obtained from Table 21 are represented in the following figure:

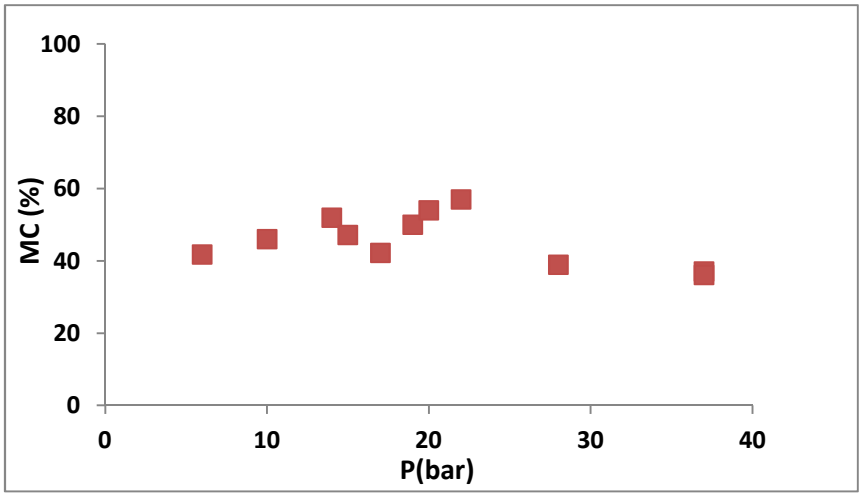


Figure 21. Results obtained from PHB Cells with 8 mL methanol.

As it can be seen in Figure 21, the results obtained show that MC conversion keeps almost constant (from 47% to 57%) at different pressures until 27 bar when the conversion starts to go down because of the same reason as before with pure PHB, but now the MC conversion doesn't seem to decrease dramatically as with pure PHB.

The same experiment was done with 10 mL methanol with PHB Cells at different pressures. The results are as follows:

Table 22. Results from PHB Cells with 10mL methanol at different pressures.

Entry	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P bar)
1	43	13	5	39	8
2	47	3	2	48	11
3	47	4	-	49	11
4	43	4	5	48	12
5	51	2	3	45	16
6	48	3	-	49	16
7	49	2	5	44	19
8	50	2	-	47	21
9	37	5	33	25	25
10	45	4	32	19	26
11	47	3	22	28	29
12	38	5	49	9	33

Reaction conditions: T=200°C, t=6h, PHB Cells (0.600g PHB), 10 mL methanol, no 3HB (%) was observed. Results from JHO14 (PHBC B) and JHO20 (PHBC C).

The results obtained in Tables 20, 21 and 22 are shown in Figure 16:

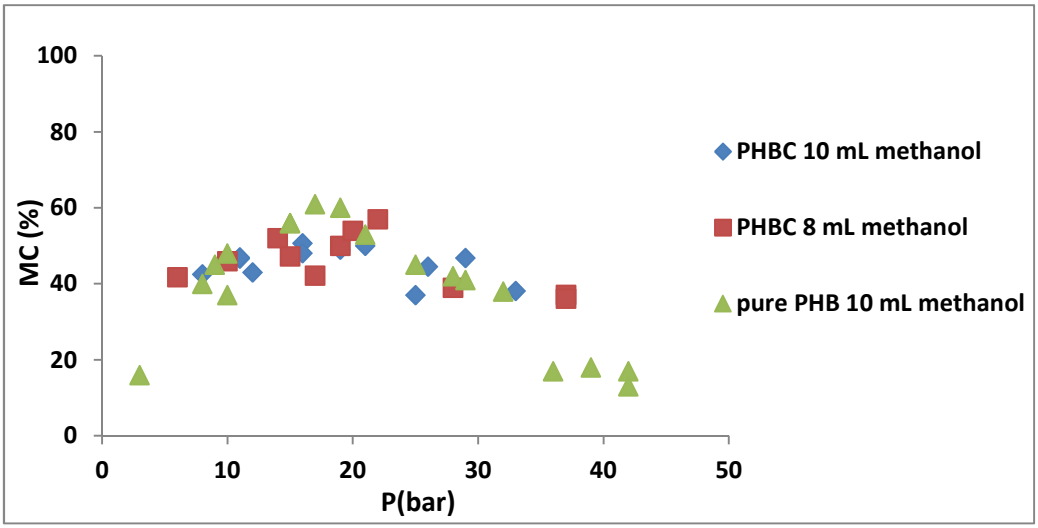


Figure 22. MC conversion in pure PHB and PHB Cells at different pressures.

As it can be seen in Figure 22, there isn't a big difference between the results obtained with PHB Cells at 8mL and 10 mL of methanol, conversions are from 40% to 50% until high pressure of 36 bar, when the conversions go down.

Comparing now PHB Cells with pure PHB, the main differences are that with PHB Cells, MC conversion is more constant until pressures of 30 bar being around 50%, while with pure PHB there is clearly a peak at pressures from 15 bar to 20 bar and then a big decrease as the pressure goes up when the methanol does no longer react with the CA. At high pressures PHB Cells show less conversion than before, from 50% to 38% but not so significantly as with pure PHB where there is a big decrease from 50% to 17%. Some of the components that are in the samples of PHB Cells give the reaction the chance to take place at high pressures without showing a very different conversion and then letting the methanol react with CA place longer than samples of pure PHB, although we expect that it will also go down at higher pressures but not so drastically as with pure PHB.

The same experiment has been done at different pressures, but this time using a catalyst of Mg $(OH)_2$. The results are as follows in Table 23:

Table 23. Results obtained from JHO21 and JHO22. Pure PHB with $Mg(OH)_2$ as a catalyst. Different pressures

Entry	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
1	40	37	2	21	7
2	55	18	11	17	8
3	58	5	3	34	16
4	57	6	-	38	16
5	47	1	5	47	36
6	48	2	1	48	33
7	43	1	7	48	34

Reaction conditions: T=200 °C, T=6h, 40 mg catalyst (10%mol), 10 mL methanol, 0.600g pure PHB, no 3HB (%) was observed.

The results obtained compared to previous experiments with PHB Cells and pure PHB are presented in the following figure:

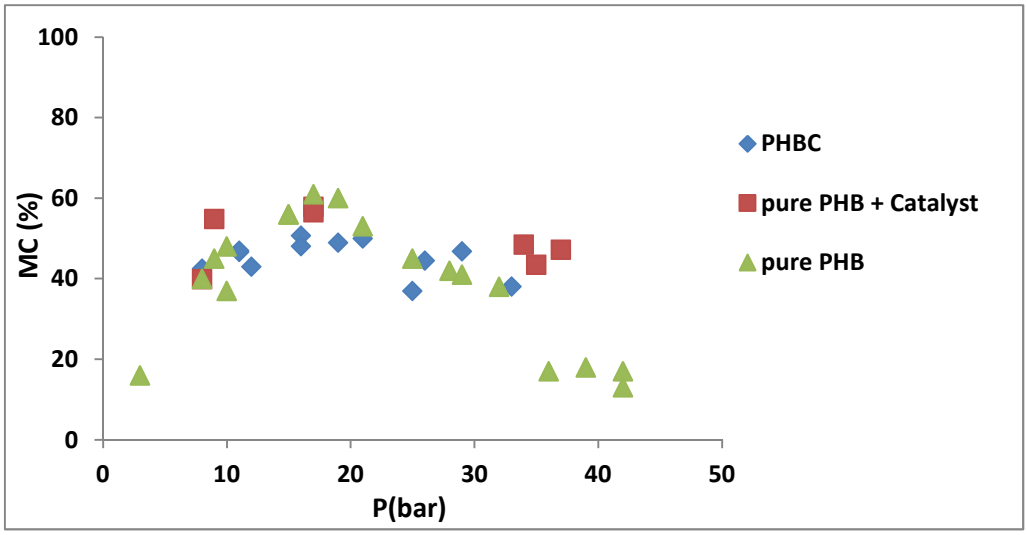


Figure 23. MC conversion in pure PHB and PHB Cells at different pressures. All experiments with 10 mL methanol.

As it has been explained previously in the experiment done with pure PHB with catalyst at different residence times, the Lewis acidity makes the conversion of MC get higher faster than with pure PHB and as it can be seen in figure 23, also gives the chance to work under higher pressures without losing to much conversion as with pure PHB.

As it can be seen in Table 23 and Figure 23, MC conversions with the catalyst are slightly higher than with just pure PHB and doesn't show a pick at pressures between 15 bar and 20 bar.

The biggest difference can be seen when the pressure reaches high values from 30 bar to 35 bar where it has a MC conversion from 40% to 50% while with just pure PHB it reaches conversions of 17% and 18%. It's sure to say then that the presence of nutrients like Mg makes the reaction be able to take place also under high pressures and get higher conversions than just using purified PHB.

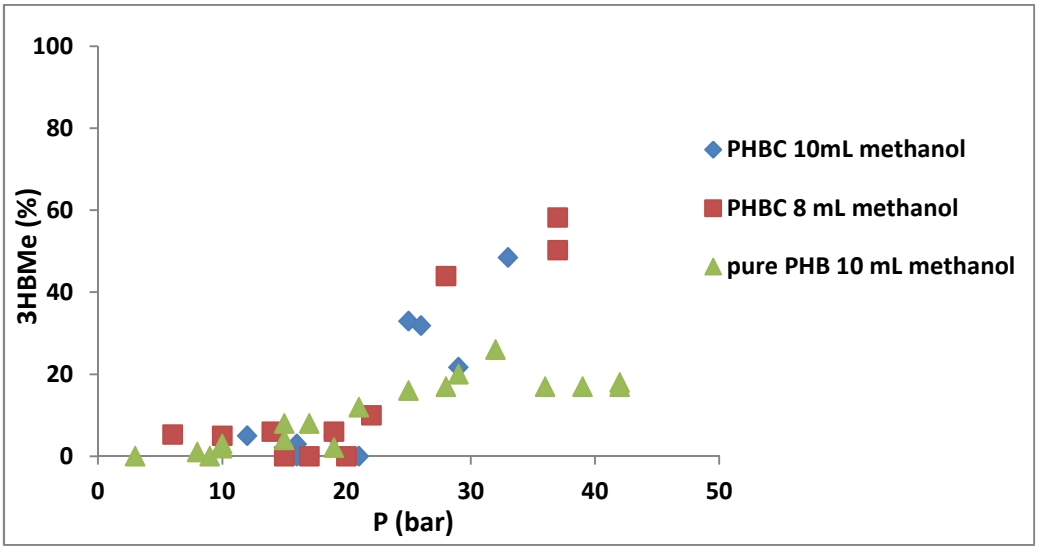


Figure 24. 3HBMe conversion in pure PHB and PHB Cells at different pressures.

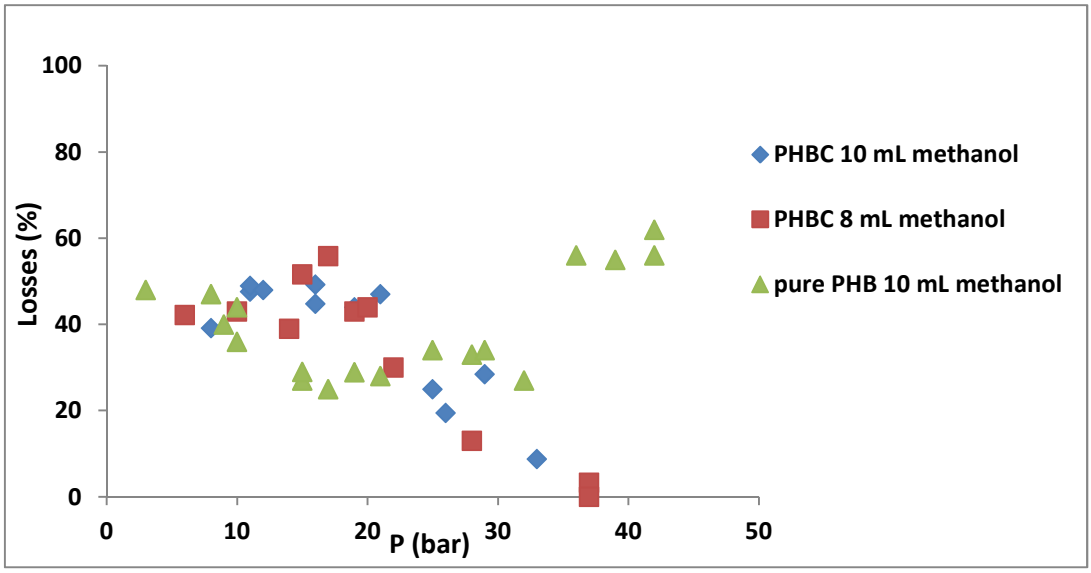


Figure 25. Losses production in pure PHB and PHB Cells at different pressures.

In Figure 24, it is shown the 3HBMe conversion at different pressures in the same reactions shown before in Tables 20, 21 and 22.

In the first part of the graph, until 20 bar, the 3HBMe conversion and losses are similar in both PHB Cells and pure PHB, but when the pressure rises from 20 bar there is a clear different behaviour in the reaction. When working with pure PHB at pressures higher than 20 bar, MC conversion goes down and 3HBMe stays constant, while the losses in form of gases increase (Figure 24). In PHB Cells, at high pressures, instead of increasing losses as MC goes down there is an increase in 3HBMe. At 36 bar, 3HBMe conversion for pure PHB is 17% while with PHB Cells is 58%.

The results show that when the samples are used from PHB Cells and PHBV20, with percentages of valerate of 16% and 20%, the 3HBMe conversion goes up at high pressures while with pure PHB (2% valerate) goes down. The presence of valerate, according to [16], makes the sample less crystalline and then have more amorphous regions than the purified PHB, methanol reacts with the components that are in the bottom of the reactor instead of the CA forming more 3HBMe while with samples of pure PHB the decarboxylation of PHB is faster forming CO and CO_2 instead of 3HBMe.

To see if this hypothesis was true, the results obtained from pure PHB + catalyst were compared to the ones obtained from Tables 21, 22 to see if the formation at high pressures of 3HBMe was due to the presence of valerate or the presence of some nutrients in the samples from PHB Cells. The results are presented in Figure 26.

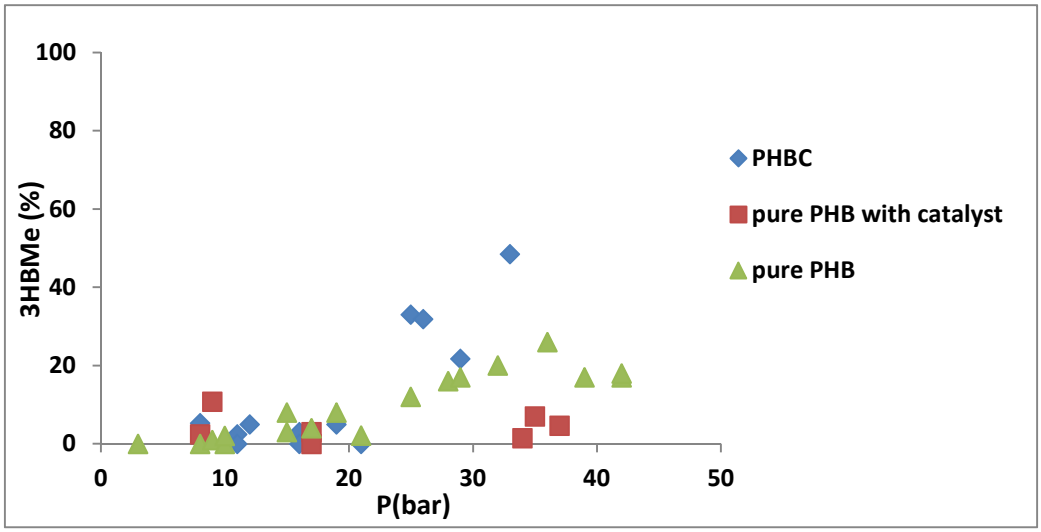


Figure 26. 3HBMe conversion in pure PHB with and without catalyst and PHB Cells. All with 10 mL methanol.

In Figure 26, it is shown that the conversion of 3HBMe has similar values in pure PHB with and without catalyst, so the hypothesis shown before is true and the high conversion of 3HBMe at higher pressures than 30 bar is due to the presence of valerate which makes reaction of PHB with the methanol faster because the valerate makes the samples of PHB Cells less crystalline than samples of pure PHB.

5. Conclusions

The aim of this master thesis was to see if the reaction to obtain methyl crotonate could be done taking as raw material PHB directly from the fermentation process instead of purified PHB. The reaction involving PHB with biomass at reaction conditions of T=200°C, 10 mL methanol, P=15-20bar, t=6h showed similar conversions (49%) to the ones obtained with pure PHB (53%).

In both samples it is observed a decrease in MC conversion under aqueous conditions, rising the conversion of 3HBMe in the case of PHB Cells and the losses in the case of pure PHB. The higher presence of valerate (16%) in PHB Cells compared to pure PHB (2%) is the responsible of this behaviour, making the sample less crystalline and then easier to react with the methanol and obtain 3HBMe as by-product instead of turning into gases like CO,CO_2.

The presence of nutrients from the fermentation stock in the samples of PHB Cells show that the reaction takes place faster than with pure PHB, being 3h and 6h respectively the required time to reach the optimal conversion (48% to 53%) in both cases.

The results done with $Mg(OH)_2$ as catalyst, support this theory making the conversion go higher to 60% at a residence time of 3h in samples with pure PHB. More research should be done to see which one of the nutrients from the fermentation stock has more effect on MC conversion but it has been demonstrated that working with PHB Cells is a viable alternative to purified PHB

6. Recommendations

The difference in the behaviour of PHB Cells and pure PHB can be due to the residues from the fermentation stock and also because the morphology PHB present in the samples.

I would recommend the use of cells without PHB presence and then adding purified PHB to see if the difference of both reactions at high pressures is because of the presence of residues from fermentation and biomass or because of the morphology of PHB, which can be different in the purified product and in the PHB present in the cells from Mars company.

The presence of residues from the fermentation clearly affects the conversion of MC as we have seen in the experiments done with $Mg(OH)_2$. There was only enough time to do some experiments with pure PHB with this substance, so I would recommend to study also the presence of $Mg(SO_4)_2$ to see which has more repercussion in the reaction, if the presence of ion OH^- or the presence of cation Mg^{2+}. The fermentation stock also contained KH_2PO_4 and KCl, so it would be also interesting to study if the effect of K^+ raises MC conversion higher than with the use of $Mg(OH)_2$ or $Mg(SO_4)_2$.

The presence of valerate in the samples of PHB Cells and PHBV20 have demonstrated a higher conversion of 3HBMe at high pressures than with pure PHB. To see if the high conversion of 3HBMe at high pressures in these samples is due to the presence of valerate, I would recommend the use of samples of PHBV with a percentage of valerate more than 20%.

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9. Appendix

- **Experiments**

JHO01 (pure PHB)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)
pure PHB	54	1	3	45

Reaction conditions: 0.600g pure PHB, T=200°C, t= 6h, 10 mL methanol, no 3HB (%).

JHO02 (PHB Cells)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)
Sample A	53	3	3	41
Sample B	46	2	7	45
Sample D	45	3	8	43
Sample C	46	13	16	24

Reaction conditions: 0.600g pure PHB (PHB Cells), T=200°C, t= 6h, 10 mL methanol, no 3HB (%).

JHO03 (PHB Cells + water)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)
pure PHB +water	8	43	29	17
Sample A + water	5	24	16	13
Sample B + water	22	10	5	34
Sample D + water	30	2	-	60

Reaction conditions: T=200 °C, t= 6h, PHB Cells (0.600g PHB), 10 mL of methanol. Water experiments with 10 mL, no 3HB (%).

JHO04 (PHB Cells)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)
Sample A	49	2	18	31
Sample B	57	2	5	36
Sample C	52	1	6	41
Sample C	50	3	9	38
Pure PHB	54	5	13	28

Reaction conditions: T=200 °C, t= 6h, PHB Cells (0.600g PHB), 10 mL of methanol, no 3HB (%).

JHO06 (Residence time, PHB Cells and PHBV)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
Sample D 4h	48	4	20	29	24
Sample D 5h	44	9	-	48	11
Sample D 6h	48	2	14	36	27
Sample D 7h	46	1	4	49	25
Sample D 8h	45	2	1	52	20
SAMPLE PHBV 6h	60	5	14	21	23

Reaction conditions: T=200°C, 10 mL methanol, Sample D PHB Cells (0.600g PHB), no 3HB (%).

JHO07 (Different amount of methanol, PHB Cells and PHBV)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
Sample D + 20 mL methanol	36	5	20	39	30
Sample D + 15 mL methanol	42	4	6	48	17
Sample D + 5 mL methanol	47	1	1	51	16
0.600 g PHBV + 15 mL methanol	32	19	15	34	27
1.200 g PHBV + 10 mL methanol	64	5	6	25	20
2.400 g PHBV + 10 mL methanol	59	22	6	13	17

T=200 °C, t= 6h, Sample D PHB Cells (0.600g PHB), no 3HB (%).

JHO08 (Water, PHB Cells)

	MC (%)	CA (%)	3HB (%)	3HBMe (%)	Losses (%)	P (bar)
Sample C + 2mL Water	41	6	8	32	13	21
Sample C + 4mL Water	13	12	10	31	34	20
Sample C + 6mL Water	14	16	13	38	19	21
Sample C + 8mL Water	16	19	15	43	7	21
Sample C + 10mL Water	12	25	17	39	7	20
Sample PHBV + 10 mL Water	3	30	20	49	-	21

Reaction conditions: T=200 °C, t= 6h, 10 mL of methanol, Sample C PHB Cells (0.600g PHB) and PHBV20 (0.600g PHB).

JHO10 (Different amount of PHB with different amount of water, pure PHB)

	MC (%)	CA (%)	3HB (%)	3HBMe (%)	Losses (%)	P (bar)
1.800g pure PHB + 2mL water	38.6	22.2	9.5	5.8	23.9	20
1.800g pure PHB + 4mL water	22.9	46.2	3	1	26.8	16
1.800g pure PHB + 7mL water	16.5	28.6	10	3	42	22
0.300g pure PHB + 2mL water	17.1	33.4	-	3	46.5	23
0.300g pure PHB + 4mL water	17	31.6	14.3	-	37.1	22
0.300g pure PHB + 7mL water	8.6	36.5	24.3	4.3	26.3	19

Reaction conditions: T=200 °C, t= 6h, 10 mL of methanol, 0.600g PHB.

JHO11 (PHB Cells and different amount of methanol, PHB Cells and pure PHB)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
Sample A + 10mL methanol	30	2	15	54	20
Sample B + 10mL methanol	49	3	5	43	16
Sample C + 10mL methanol	49	1	7	43	22
Sample D + 10mL methanol	44	2	-	54	19
pure PHB + 15 mL methanol	12	13	13	63	29
pure PHB + 5 mL methanol	57	5	7	32	16

Reaction conditions: T=200 °C, t= 6h, Sample D PHB Cells (0.600g PHB), no 3HB(%).

JHO12 (Residence time, PHB Cells)

	MC (%)	CA (%)	% 3HB (%)	3HBMe (%)	Losses (%)	P (bar)
Sample B 1h	28	26	4	20	22	13
Sample B 2h	37	10	-	16	37	14
Sample B 3h	47	2	-	5	45	13
Sample B 4h	47	5	-	6	43	14
Sample B 5h	43	3	3	19	32	22
Sample A 6h (JHO11)	35	1	-	6	58	21

Reaction conditions: T=200°C, 10 mL methanol, Sample B PHB Cells (0.600g PHB).

JHO13 (Different amounts of methanol, PHB Cells)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
Sample B + 4mL methanol	42	4	4	50	12
Sample B + 5mL methanol	45	3	3	50	15
Sample B + 6mL methanol	43	1	-	56	17
Sample B + 7mL methanol	50	1	-	48	16
Sample B + 8mL methanol	59	2	4	35	18
Sample B + 9mL methanol	53	1	5	41	19

Reaction conditions: T=200°C, t=6h, Sample B PHB Cells (0.600g PHB), no 3HB (%).

JHO14 (Different pressures with 10 mL methanol, PHB Cells)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
Sample B	43	4	5	48	12
Sample B	47	4	-	49	11
Sample B	49	2	5	44	19
Sample B	50	2	-	47	21
Sample B	45	4	32	19	26
Sample B	37	5	33	25	25

Reaction conditions: T=200°C, t=6h, 10 mL methanol, Sample B PHB Cells (0.600g PHB), no 3HB (%).

JHO15 (Temperatures, PHB Cells)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
Sample B T=180 °C	41	12	20	28	10
Sample B T=180 °C	38	10	21	32	12
Sample B T=200 °C	51	1	3	45	17
Sample B T=200 °C	53	2	-	45	16
Sample B T=220 °C	38	10	2	50	15
Sample B T=220 °C	43	0	-	56	19

Reaction conditions: t=6h, 10 mL methanol, Sample B PHB Cells (0.600g PHB), no 3HB (%).

JHO16 (Different pressures 8 mL methanol, PHB Cells)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
Sample B	46	6	5	43	10
Sample B	52	2	6	39	14
Sample B	50	1	6	43	19
Sample B	54	3	-	44	20
Sample B	51	3	10	36	22
Sample B	39	5	44	13	28

Reaction conditions: T=200°C , t=6h, 8 mL methanol, Sample B PHB Cells (0.600g PHB), no 3HB (%).

JHO17 (Different amounts of methanol, PHBV)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
PHBV + 5mL methanol	48	18	2	33	8
PHBV + 7mL methanol	59	11	4	26	15
PHBV + 9mL methanol	66	6	-	28	15
PHBV + 5mL methanol	43	4	-	53	15
PHBV + 7mL methanol	59	10	5	27	13
PHBV + 9mL methanol	64	9	3	23	14

Reaction conditions: T=200°C , t=6h, PHBV (0.600g PHB), no 3HB (%).

JHO18 (Residence time, PHB Cells)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
Sample C 1h	33	20	26	21	15
Sample C 2h	44	10	10	36	17
Sample C 3h	44	3	7	46	18
Sample C 5h	52	4	-	43	19
Sample C 7h	50	1	4	46	20
Sample C 8h	49	0	5	45	20

Reaction conditions: T=200°C, 10 mL methanol, Sample C PHB Cells (0.600g PHB).

JHO19 (Different pressures 8 mL methanol, PHB Cells)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
Sample C	42	11	5	42	6
Sample C	42	2	-	56	17
Sample C	47	1	-	52	15
Sample C	30	9	50	10	37
Sample C	36	10	58	-	37

Reaction conditions: T=200°C, t=6h, 8 mL methanol, Sample C PHB Cells (0.600g PHB), no 3HB (%).

JHO20 (Different pressures 10 mL methanol, PHB Cells)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
Sample C	14	4	2	81	8
Sample C	21	1	1	77	11
Sample C	20	1	1	78	16
Sample C	31	2	-	68	16
Sample C	34	2	16	48	29
Sample C	33	4	42	21	33

Reaction conditions: T=200°C, t=6h, 10 mL methanol, Sample C PHB Cells (0.600g PHB), no 3HB (%).

JHO21 (Water and catalyst, PHB Cells and pure PHB)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
Sample C + 2mL water	41	9	30	20	19
Sample C + 4mL water	30	12	30	28	22
Sample C + 6mL water	17	22	39	22	21
Sample C + 8mL water	12	27	-	61	20
Sample C + 10mL water	12	26	45	17	23
pure PHB + Mg(OH)2	48	2	1	48	34

Reaction conditions: T=200°C, t=6h, 10 mL methanol, Sample C PHB Cells (0.600g PHB), no 3HB (%). Catalyst (40 mg $Mg(OH)_2$, 10 %mol).

JHO22 (Different pressures, catalyst pure PHB)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
pure PHB + Mg(OH)2	40	37	2	21	8
pure PHB + Mg(OH)2	55	18	11	17	9
pure PHB + Mg(OH)2	58	5	3	34	17
pure PHB + Mg(OH)2	57	6	-	38	17
pure PHB + Mg(OH)2	47	1	5	47	37
pure PHB + Mg(OH)2	48	2	1	48	34
pure PHB + Mg(OH)2	43	1	7	48	35

Reaction conditions: T=200°C, t=6h, 10 mL methanol, 0.600g pure PHB, no 3HB (%). Catalyst (40 mg $Mg(OH)_2$, 10 % mol).

JHO23 (Residence time, catalyst pure PHB)

	MC (%)	CA (%)	3HBMe (%)	Losses (%)	P (bar)
pure PHB + Mg(OH)2 t= 1h	47	21	4	28	18
pure PHB + Mg(OH)2 t= 1h	36	35	4	25	16
pure PHB + Mg(OH)2 t= 2h	49	17	5	29	19
pure PHB + Mg(OH)2 t= 2h	58	15	-	28	15
pure PHB + Mg(OH)2 t= 3h	37	35	5	23	11
pure PHB + Mg(OH)2 t= 3h	60	5	2	34	18

Reaction conditions: T=200°C, 10 mL methanol, 0.600g pure PHB, no 3HB (%). Catalyst (40 mg $Mg(OH)_2$, 10 %mol).

- **PHA content and substrate (cells Delft University)**

	Date	Total COD mgCOD/l	soluble COD mgCOD/l	Acetate mgCOD/l	Propionate mgCOD/l	Butyrate mgCOD/l	Valerate mgCOD/l
Sample A	16.10.2013	11242	10658	2072	937	2203	268
Sample B	22.10.2013	9764	9338	2113	1099	1840	378
Sample C	26.11.2013	9252	8564	1582	718	2030	479
Sample D	11.12.2013		5392	1671	550	1561	284

Hexanoate mgCOD/l	Ethanol mgCOD/l	Other identified mgCOD/l	unidentified	VFA	VFA + EtOH	Other sCOD
1273	3153	0	752	6753	9906	752
1330	2041	0	537	6760	8801	537
1376	2302	0	77	6185	8487	77
0	994	169	164	1066	5059	333

- **TGA**

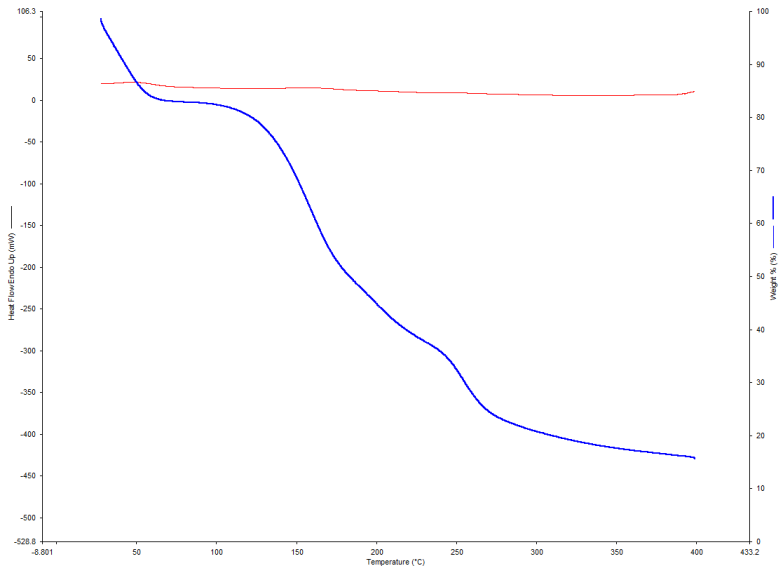

Figure 1. TGA for Sample C PHB Cells.

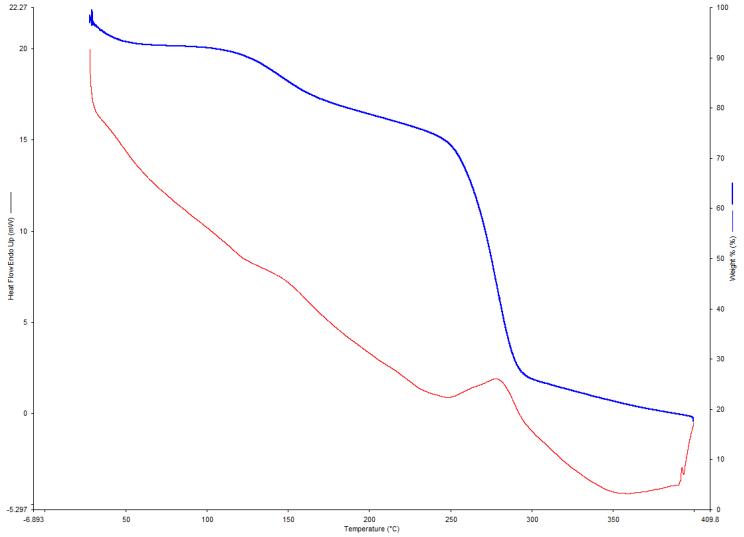

Figure 2. TGA for Sample B PHB Cells.