PRODUCTION OF MEDIUM CHAIN LENGTH POLYHYDROXYALKANOATES BY *Pseudomonas fluorescens* AND UNRELATED CARBON SOURCE

DIANA MARCELA VANEGAS HERNÁNDEZ
Ph.D, Chemical engineer

MARGARITA ENID RAMÍREZ CARMONA
Ph.D, M.Sc, Chemical engineer

Universidad Pontificia Bolivariana

INTRODUCTION

Universidad Pontificia Bolivariana

PRODUCTION

260 millons of tons

COST EFFECTIVENESS

Glucose→ 9,5 $USD/kg

Producción cost→ 40-48% raw materials

PROBLEM ENVIRONMENTAL

polluting waste

Depletion of natural resources

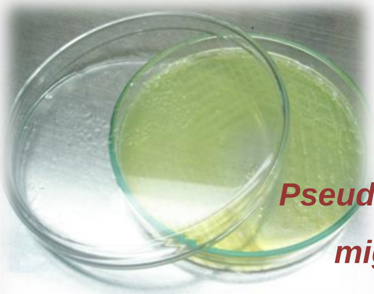
1% of plastics global demand

90% of world consumption PLA, PHA

Annual growth rate of 50.7%

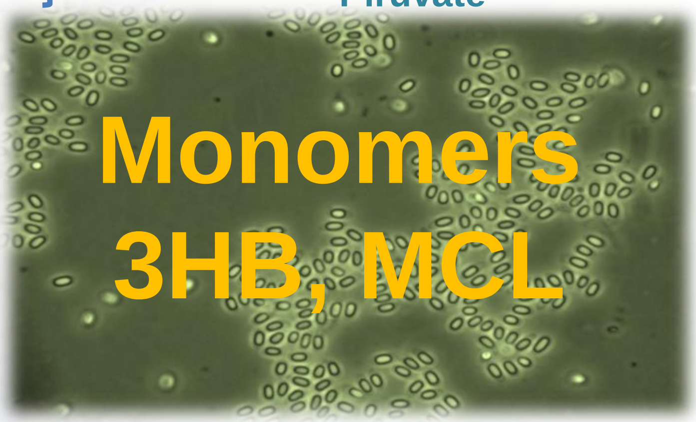
INTRODUCTION

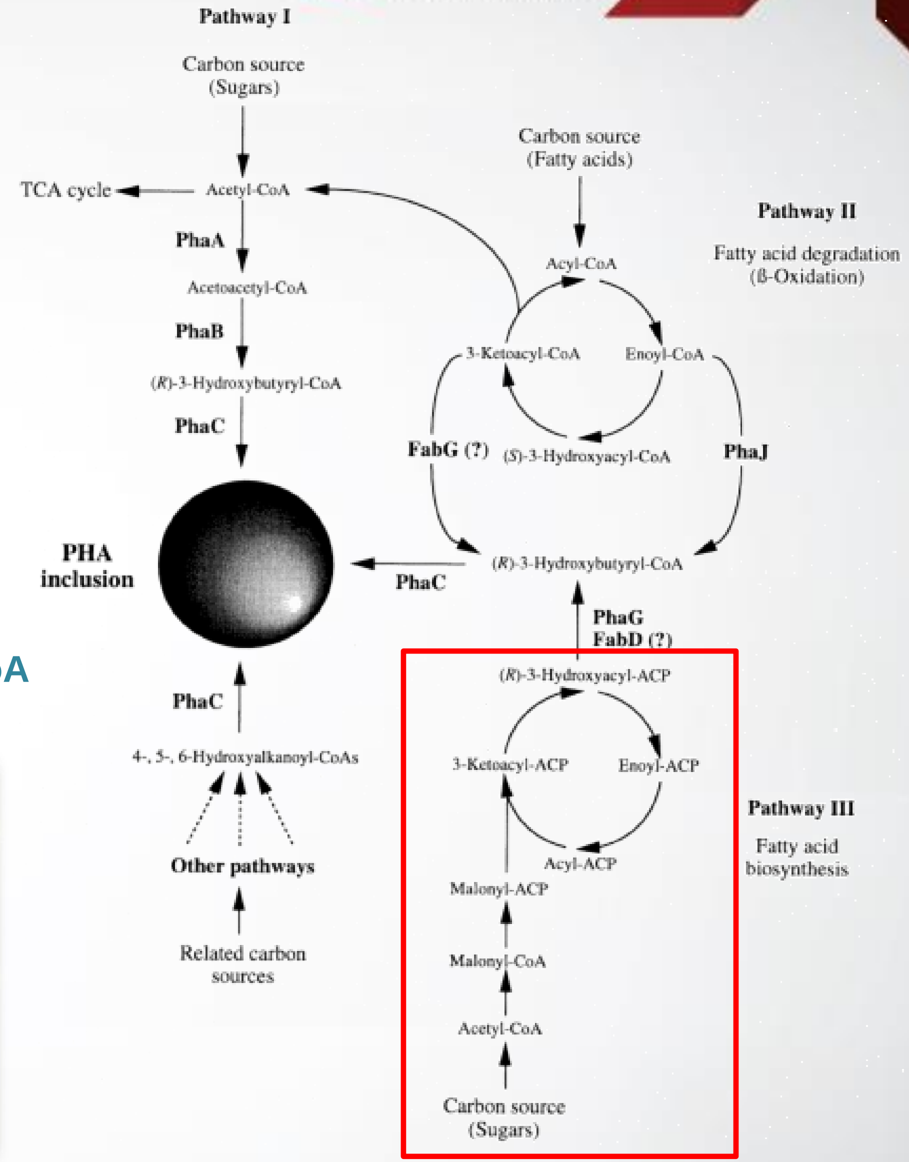
1. MICROORGANISM



Pseudomonas fluorescens migula ATCC 49838

GLUCOSE – Phosphorylation → Piruvate → Acetyl CoA





PHA synthesys methabolic pathway

METHODOLOGY

1. MICROORGANISM

Pseudomonas fluorescens migula ATCC (American Type Culture Collection) 49838

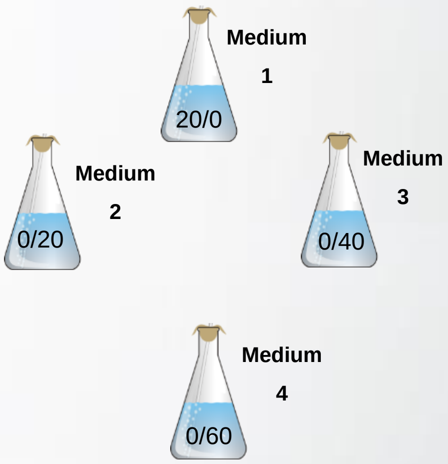
2. OPERATIIONS CONDITIONS AND CULTURE MEDIUM

Chemical analysis:

Ash, moisture, sugars, mineral components and protein.

Table 1. CULTURE BROTH

COMPONENTS	CONCENTRATION	
$(NH_4)SO_4$	1 g/L	
KH_2PO_4	1,5 g/L	
$MgSO_4.7H_2O$	1 mL/L de sln 20% (p/v)	
$CaCl_2.2H_2O$	1 mL/L de sln 1% (p/v)	
Micronutrients 1 mL/L	Components/L sln +1 mol/L de HCl	amount (mg)
	$ZnSO_4.7H_2O$	100
	$MnCl_2.4H_2O$	30
	H_3BO_3	300
	$CoCl_2.6H_2O$	200
	$CuSO_4.5H_2O$	10
	$NaMoO_4.2H_2O$	30



30 °C, 60 h; 120 rpm

METHODOLOGY

Universidad Pontificia Bolivariana

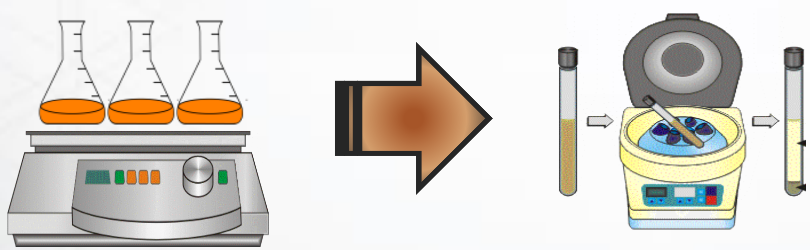
4. SELECTION OF NITROGEN SOURCE IN THE PHA PRODUCTION BY FACTORIAL DESIGN

Table 2. FACTORS AND LEVELS IN THE FACTORIAL DESIGN

Parameter	Culture medium volume		0,25 L	
	pH initial		5,5	
	Stirring speed		150 rpm	
	Fermentation time		96 h	
Factor	**UREA**		**AMONIUM SULPHATE**	
	Lower level	Higher level	Lower level	Higher level
Nitrogen (g/L)	0,24	0,65	0,5	1,5
T (ºC)	26	32	26	32
Molasses (g/L)	20	40	20	40

FACTORIAL DESIGN 2^3

FERMENTATION PROCESS



Biomass UV/VIS

PHA (dry weight)

FTIR

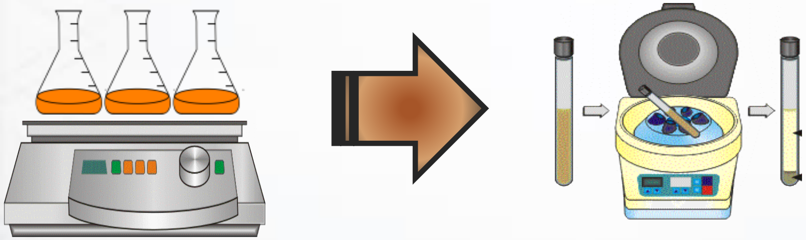
METHODOLOGY

5. OPTIMIZATION OF OPERATION VARIABLES FOR PHA PRODUCTION BY RESPONSE SURFACE DESIGN

Table 3. RESPONSE SURFACE DESIGN

Parameter	Culture medium volume	0,25 L	
	pH initial	5,5	
	Stirring speed	150 rpm	
	Fermentation time	96 h	
LEVELS	**FACTORS**		
	T (°C)	**Nitrogen (g/L)**	**Molasses (g/L)**
Higher	28	0.80	48
Lower	24	0.50	40
Central	26	0.65	44

FERMENTATION PROCESS



Biomass UV/VIS

PHA (dry weight)

FTIR

METHODOLOGY

6. EVALUACTION TIME FERMENTATION EFFECT IN THE PHA PRODUCTION

96 h

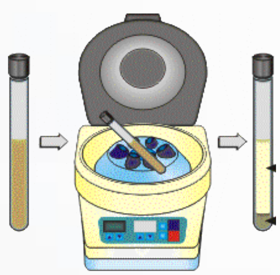
168 h

0,25 L, 150 rpm, pH 5,5

Molasses 50,7 g/L

Urea 0,9 g/L

26 °C



Biomass UV/VIS

PHA (Dry weight)

FTIR

RESULTS

Universidad Pontificia Bolivariana

2. CULTURE BROTH

Table 4. MOLASSES COMPOSITION

COMPONENTS			Comercial Molasses	Typical Molasses
Major Components	Sugars	Sacarose (%)	31	60-63
		Glucose (%)	**12**	6-9
		Fructose (%)	**13**	5-10
	Azúcares totales (%)		57	---
Protein			3,13	3

Minerals componentes → Ca, Cu, P, Fe, Mg, Mn, N, K, Na, Zn.

Fig 1. INTERVALS LEAST SIGNIFICANT DIFFERENCE

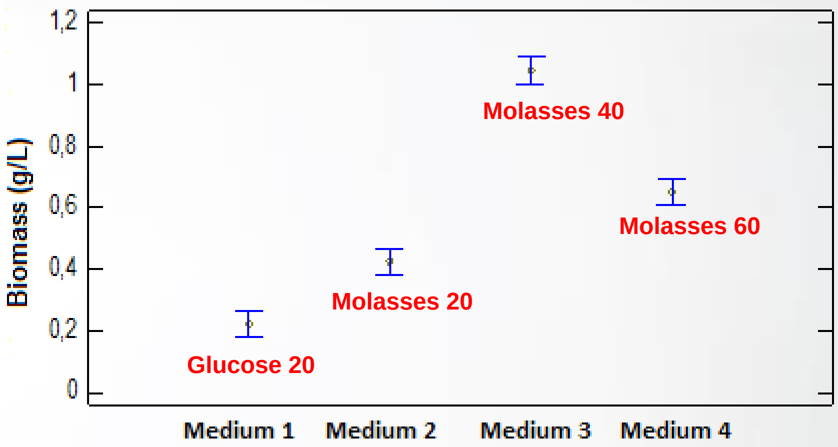


Table 5. YIELD SUBSTRATE / PRODUCT

Sustrate	Reducing sugars (g/L)	CS (%substrate conversion)	Yp/s
Molasses	10	100	**$2,37*10^{-4}$**
Glucose	20	100	$2,5*10^{-5}$

RESULTS

3. SELECTION OF NITROGEN SOURCE

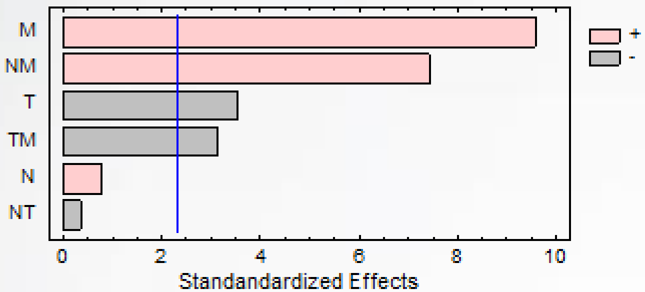


Fig 5. PARETO AMONIUM SULPHATE

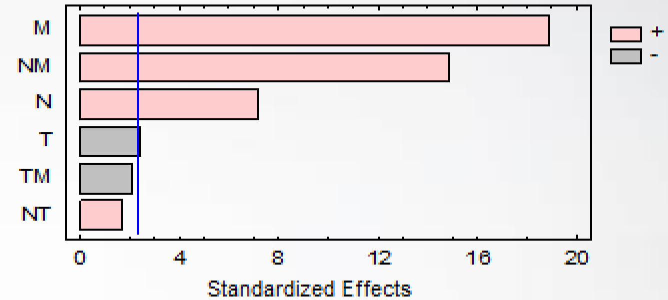


Fig 6. PARETO UREA

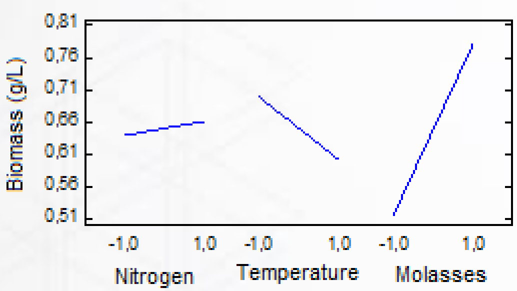


Fig 7. EFFECTS AMONIUM SULPHATE

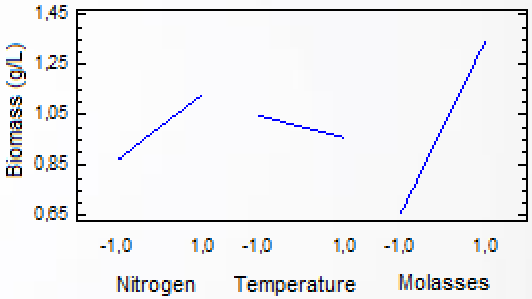


Fig 8. EFFECTS UREA

Table 6. Optimal values of the factors suggested by the design of experiments

FACTOR	OPTIMUM VALUE	
	Amonium sulphate	Urea
Nitrogen (g/L)	1,5	0,65
Molasses (g/L)	40	40
Temperature (°C)	26	26
Biomass (g/L)	0,9983	**1,7986**

RESULTS

3. SELECTION OF NITROGEN SOURCE

Table 7. EVALUATION OF POLYMER PRODUCED FROM DIFFERENT NITROGEN SOURCES

NITROGEN SOURCE	BIOMASS (g/L)	YIELD (mg PHA/ g BIOMASS)
Amonium sulphate	1,04±0,03	17,13±0,58
Urea	1,76±0,001	37,35±2,4

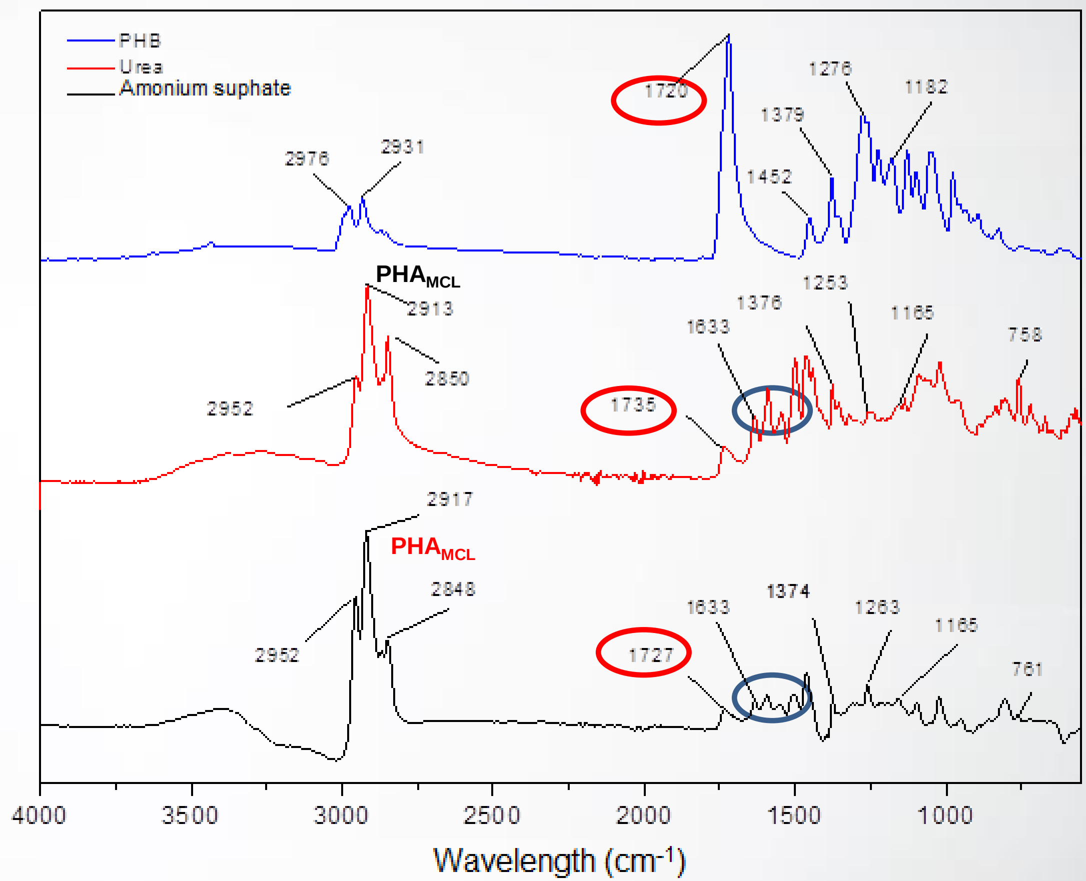


Fig 9. INFRARED OF PHB COMMERCIAL AND POLYMER OBTAINED FROM SOURCES OF NITROGEN

UREA

RESULTS

Universidad Pontificia Bolivariana

4. OPTIMIZATION OF OPERATION VARIABLES FOR PHA PRODUCTION BY RESPONSE SURFACE DESIGN

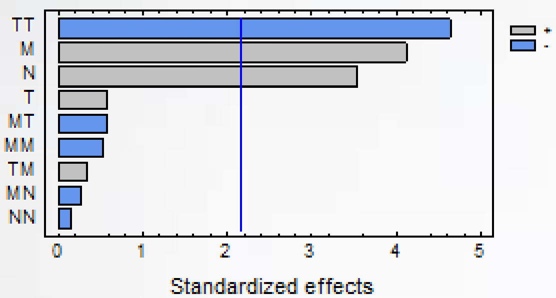


Fig 10. PARETO GROWTH BIOMASS

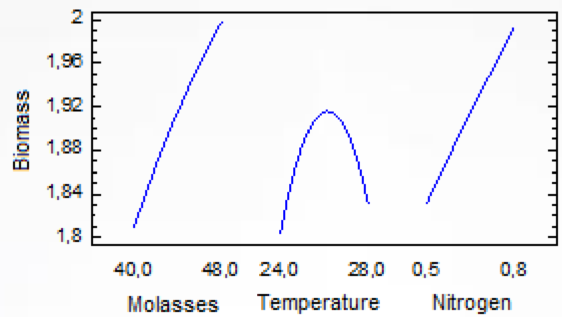


Fig 11. EFFECTS OF INDEPENDENT FACTORS IN BIOMASS CONCENTRATION

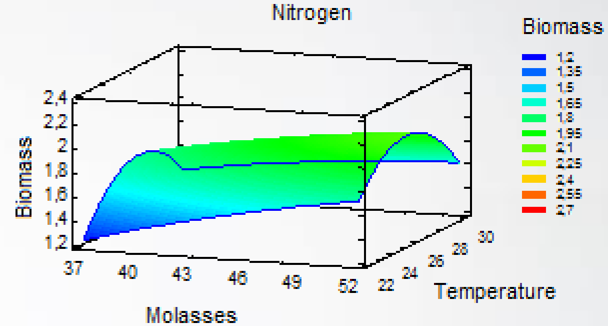


Fig 12. RESPONSE SURFACE BIOMASS CONCENTRATION

Table 8. EVALUATION OF POLYMER AFTER OPTIMIZATION

FACTOR	OPTIMUM VALUE
M (g/L)	50,70
T(°C)	26,00
N (g/L)	0,90
Biomass (g/L)	2,15

Table 9. EVALUATION OF POLYMER AFTER OPTIMIZATION

PARAMETER		BIOMASS (g/L)	mg PHA/ g BIOMASS
Urea (g/L)	0,90	2,23±0,003	29,59±2,1
Molasses (g/L)	50,70		
Temperature (°C)	26,00		

RESULTS

Universidad Pontificia Bolivariana

5. EVALUACTION TIME FERMENTATION EFFECT IN THE PHA PRODUCTION

Table 10. EVALUATION OF BIOPOLYMER TO DIFFERENT FERMENTATION TIMES

TIME	BIOMASS (g/L)	YIELD (mg PHA/ g BIOMASS)
96 h	2,23±0,003	29,59±2,1
168 h	2,07±0,24	253,27±26,26

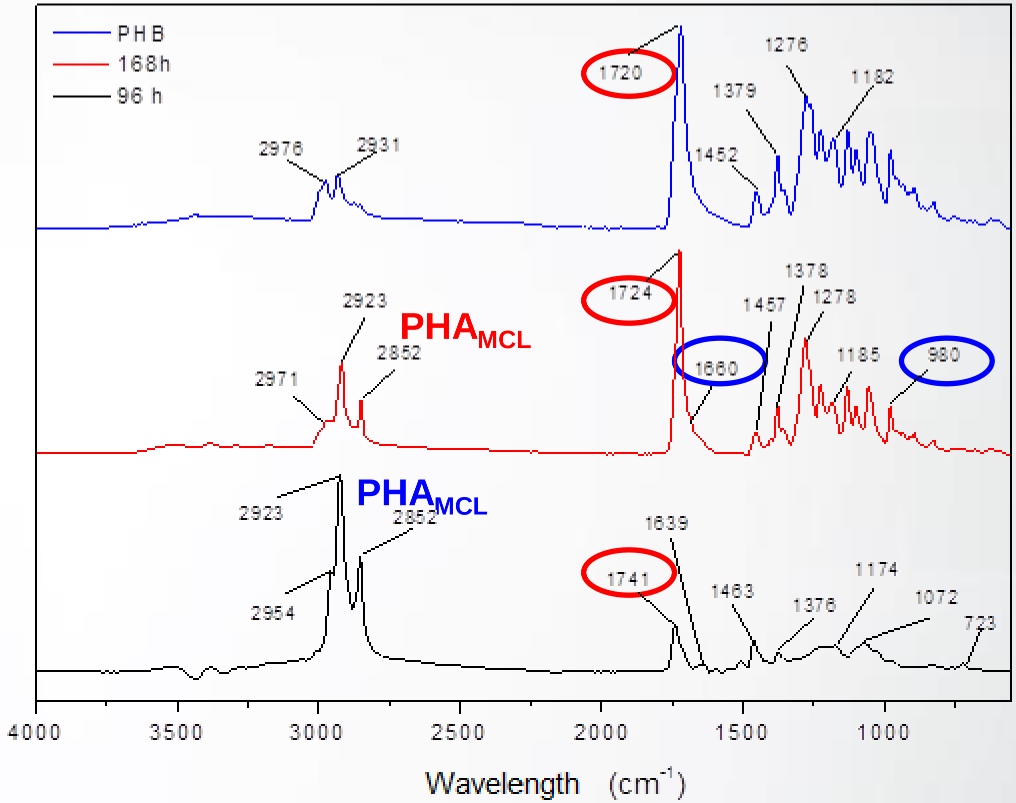


Fig 13. INFRARED SPECTRA OF BIOPOLYMER AT AT DIFFERENT FERMENTATION TIMES AND COMMERCIAL PHB

168 h

RESULTS

5. EVALUACTION TIME FERMENTATION EFFECT IN THE PHA PRODUCTION

Table 12. POLYMER COMPOSITION

FERMENTATION CONDITIONS	MONOMER					
	C4:0	C14:0	C15:0	C16:0	C18:0	C18:1
26°C 168 h	8	4	27	---	---	18
	M_W (KDA)		**M_N (KDA)**		**POLYDISPERSITY M_W/M_N**	
	998		276		3,62	

PHB

M_w 522 kDa

Polydispersity → 1,96

PHAs

M_w 1 – 10^3 kDa

RESULTS

Universidad Pontificia Bolivariana

Table 14. COMPARISON OF FEATURES BIOPOLYMER AND OTHER POLYMERS FROM MICROORGANISMS AND DIFFERENT SUBSTRATES

SUSTRATE	MICROORGANISM		MONOMER (% MOLAR)												M_W (KDA)	M_N (KDA)	MW/MN	REFERENCIA
			C4:0	C6:0	C8:0	C10:0	C12:0	C12:1	C14:0	C14:1	C15:0	C16:0	C18:0	C18:1				
Cane molasses (5 %)	*P. fluorescens*	26 °C	**8**	N.D	N.D	N.D	N.D	N.D	**4**	N.D	**27**	N.D	N.D	**18**	**998**	**276**	**3,62**	This work
Soy molasses (2 %)	*P. corrugata*		N.D	3	17	40	14	6	3	17	N.D	N.D	N.D	N.D	N.C	N.C	N.C	Solaiman, et. al, 2006
Soy molasses (5 %)	*P. corrugata*		N.D	Tr	20	49	21	Tr	Tr	12	N.D	N.D	N.D	N.D	N.C	N.C	N.C	Solaiman, et. al, 2006
Glucose (0,5 %)	*P. corrugata*		N.D	2	10	56	11	N.D	2	9	N.D	N.D	N.D	N.D	N.C	N.C	N.C	Solaiman, et. al, 2006
Oleic acid (0,5 %)	*P. corrugata*		N.D	4	42	30	10	Tr	Tr	14	N.D	N.D	N.D	N.D	N.C	N.C	N.C	Solaiman, et. al, 2006
Glycerol (5 %)	*P. corrugata*		N.D	1	14	46	9	29	1	Tr	N.D	N.D	N.D	N.D	N.C	N.C	N.C	Solaiman, et. al, 2006
Cane molasses (4 %)	*P. aeruginosa*		100	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.C	N.C	N.C	Tripathi et al., 2011
Glucose (2 %)	*P. aeruginosa*		N.D	N.D	11	74	15	N.D	N.D	N.D	N.D	N.D	N.D	N.D	122	46	2,65	Rojas-Rosas et al., 2007
Gluconate (2 %)	*recombinant R. eutropha PHB^{-4}*		100	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	162300	134500	1,22	Luo et al., 2006
Gluconate(2 %) Octanate (0,2 %)	*recombinant R. eutropha PHB^{-4}*		63	4	31	1	2	N.D	N.D	N.D	N.D	N.D	N.D	N.D	10500	7600	1,39	Luo et al., 2006
Glucose (2 %)	*P. putida*		N.D	Tr	7	74	8	9	Tr	2	N.D	N.D	N.D	N.D	N.C	N.C	N.C	Huijberts et al., 1992
Decanoate (20 mM)	*P. putida*		N.D	5	52	42	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.C	N.C	N.C	Huijberts et al., 1992

N.D: No detected; Tr: trace amounts detected; N.C: unquantified.

CONCLUSIONS

- ✓ Molasses as a substrate for polyhydroxyalkanoates production presents favorable conditions for the growth *Pseudomonas fluorescens* and promotes production of PHA_{MCL}, despite being a substrate unrelated to the length of the chains in the biopolymer produced nutrients. However, molasses concentrations around 60 g / L can inhibit the growth of the microorganism.
- ✓ Urea as a nitrogen source has conditions appropriate for *Pseudomonas fluorescens* metabolism, allowing a 40% growth over fermentations using ammonium sulfate as nitrogen source.
- ✓ The polymer from Pseudomonas fluorescens and molasses present a composition characterized by side chains mainly 15 and 18 carbons.

GRACIAS !!!!!