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Bio-hydrogen production potential of *Rhodopsuedomonas palustris* by photo-fermentation of Brewers spent grain (BSG) hydrolysate

CENTRE FOR RENEWABLE & SUSTAINABLE ENERGY STUDIES

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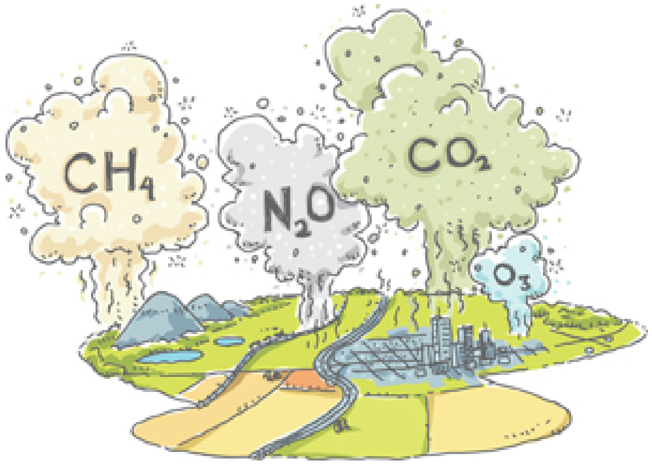
Background and motivation

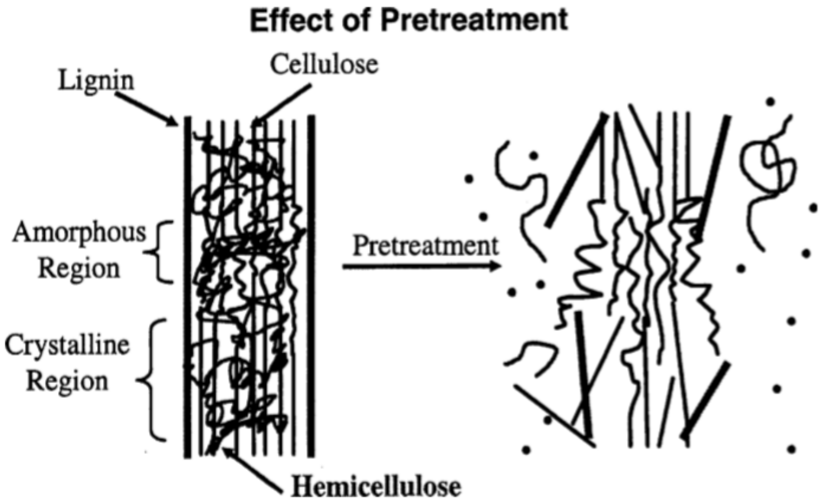
- Increasing energy demand and limited fossil fuel resources
- Greenhouse gas emissions and adverse environmental effects
- Sustainable and renewable energy development
- Lignocellulose biomass
  - Abundant (agricultural waste, industrial waste
  - Cheap biomass material
  - Renewable



Introduction

❖ Cheap and abundant resource: Lignocellulose biomass (L.C)

- Renewable resource for sustainable bio-hydrogen production
- Brewers spent grain (BSG)



- Major challenge: recalcitrance of the L.C complex to microbial degradation

❖ Lignocellulose structure and components

- Cellulose
- Hemicellulose
- Lignin

Steam explosion

- Physicochemical pre-treatment process
- Releases simple sugars from lignocellulose carbohydrate polymers
- Releases enzymatic and microbial inhibitory compounds (Short chain organic acids, phenolic compounds and furan aldehydes)
- Less energy intensive
- Environmentally friendly

- Hydrogen gas: ideal alternative energy carrier to fossil fules
  - Green fuel
  - Clean combustion (water sole product)
  - High energy content compared to hydrocarbon fuels.

- Fermentative biohydrogen (bio-H2) production processes
  - Dark fermentation
  - Photo-fermentation

- <u>Photo-fermentation (PNSB) (Anaerobic or microaerobic)</u>
  - Fermentative process
  - Photophosphorylation

- <u>*Rhodopsuedomonas palustris* CGA009</u>
  - Utilize all four modes of metabolisms
  - Nitrogen fixation and obligatory hydrogen production
  - Aromatic/phenolic degradation abilities
  - Oxidizes organic acids

Aims and objectives

Aims:

- The aim of the study is to evaluate bio-hydrogen production potential of *R. palustris* through photo-fermentative conversion of inhibitory compounds to hydrogen, while leaving simple sugars unconsumed.

- Objectives:

✓ Prepare lignocellulose hydrolysate from BSG biomass by steam explosion pretreatment.

✓ Analyze L.C hydrolysate composition

✓ Cultivate and prepare *R. palustris* CGA009 inoculum

✓ Investigate the effect of organic acids, phenol and furan compounds on growth and bio-hydrogen production of *R. palustris* CGA009 (current experiment)

✓ Ferment BSG hydrolysate at different dilutions with liquid medium (current experiment)

Materials and methods

Biomass pre-treatments: Steam explosion

Pre-treatments conditions (1) 180ºC and 10 minutes

(2) 200ºC and 5 minutes

Bacterial strain: *Rhodopsuedomonas palustris* CGA009

Growth conditions: Anaerobic and micro-aerobic conditions at 35ºC and pH 7

Analytical procedures

High Performance Liquid Chromatography analysis:

- Organic acids (acetic acid, formic acid and lactic acid)
- Phenolic compounds
- Furan derivatives (furfural and HMF)
- Simple sugars (glucose, xylose and arabinose)

Cell growth analysis

- Dry cell weight analysis: filter paper method
- Spectrophotometer absorbance at 660nm

Hydrogen production

- Volumetric hydrogen: gas-water displacement method
- Hydrogen content: gas chromatography

Substrate consumption: chemical oxygen demand (COD) analysis

Nitrogen content: Total nitrogen analytical kit

Experimental setup:

Table A

Compound name	Concentration tested (g/L)
Protocatechuate	0.076
Vanillic acid	0.107
Syringic acid	0.143
Vanillin	0.43
p-Coumaric acid	0.34
Syringaldehyde	0.03
Ferulic acid	0.14
Coniferaldehyde	0.054

Table B

Compound name	Concentrations tested (g/L)
5-hydroxymethylfurfural (HMF)	0.0965 – 1.929
Furfural	0.0634 - 1
Arabinose	5.3697
Xylose	7.05

Table C

Photofermenattion	Hydrolysate dilution ratio (%) tested				
Anaerobic and microaerobic	5	10	20	30	40

Results

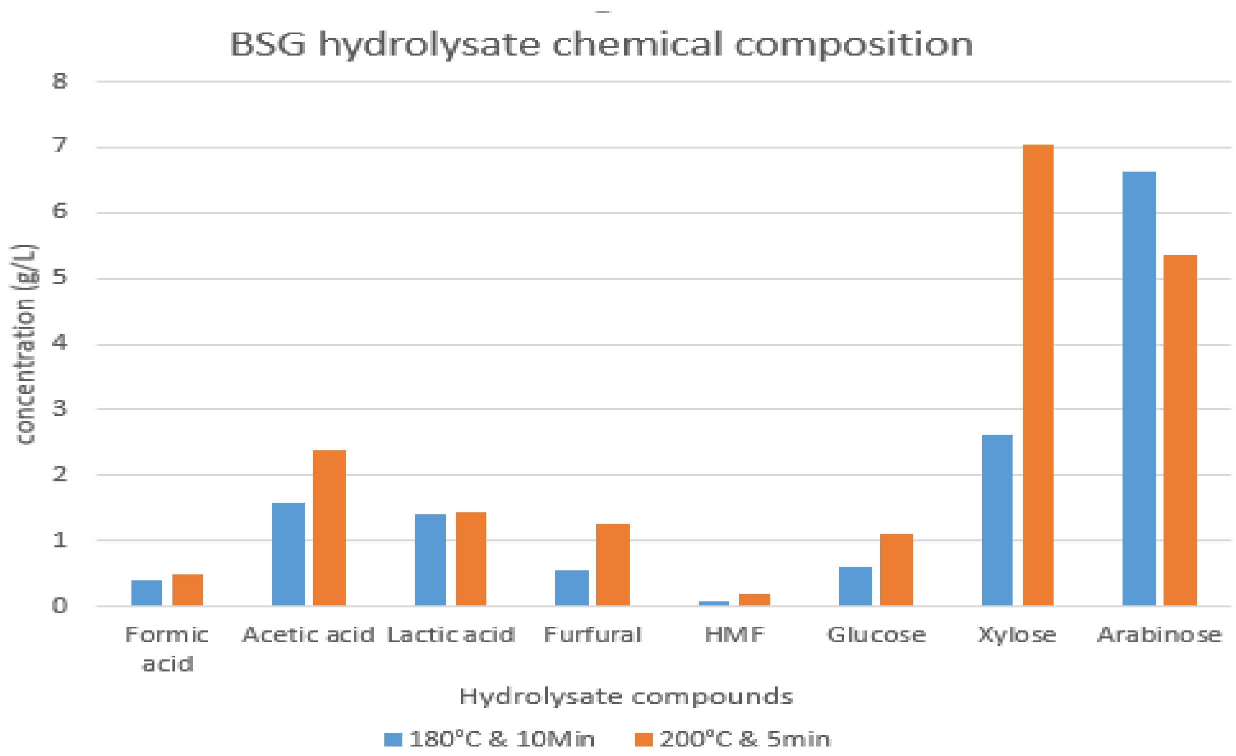


Figure 1: Chemical composition of hydrolysate prepared by steam explosion of BSG biomass.

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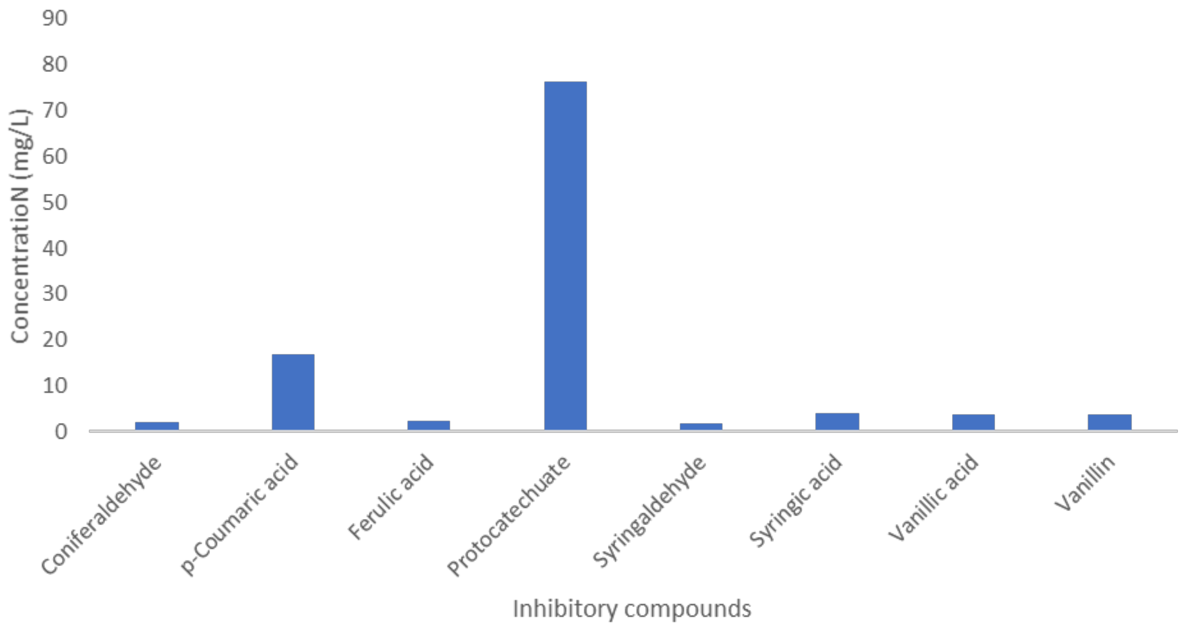

Figure 2: Chemical composition of phenolic compounds in BSG hydrolysate.

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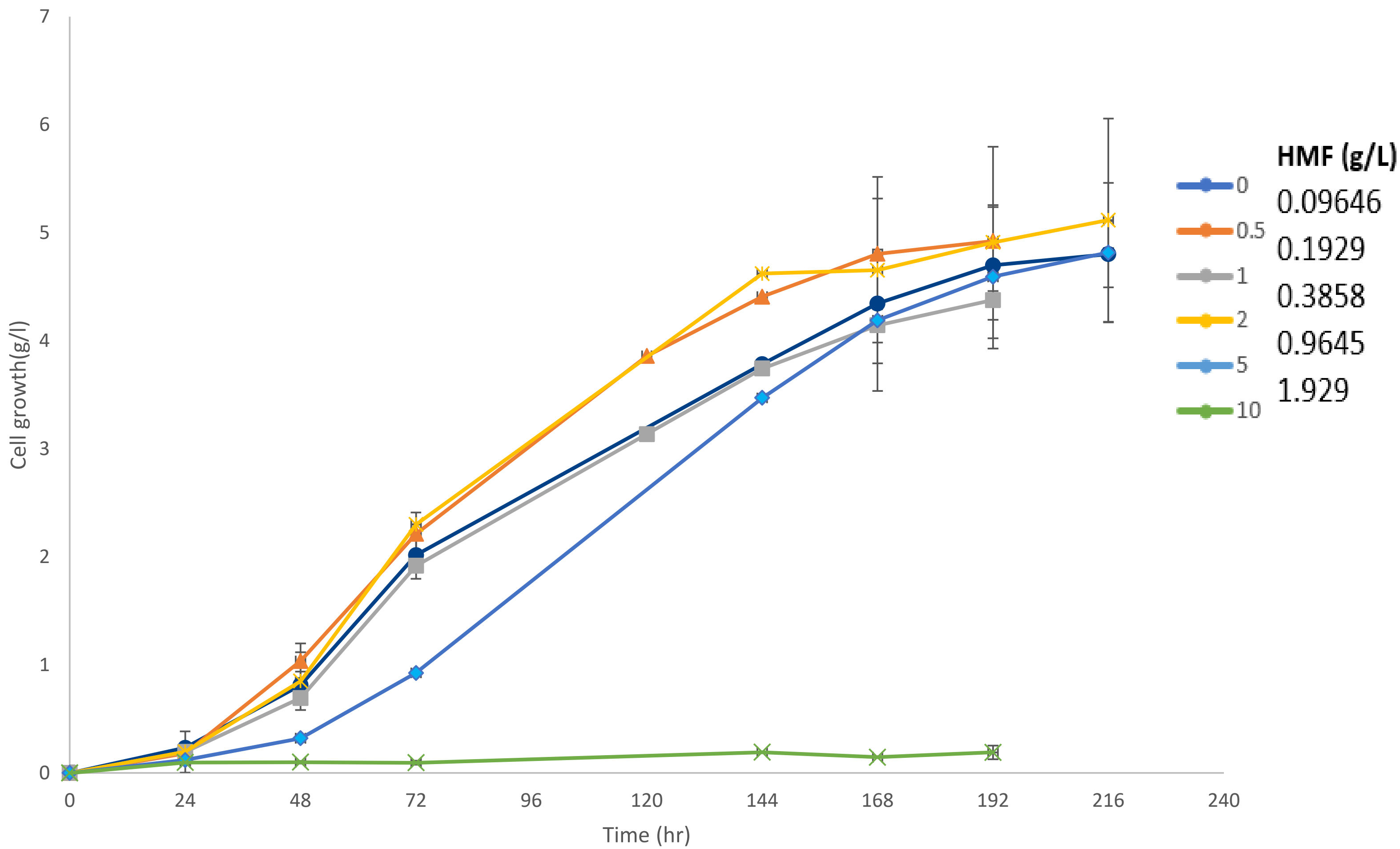

Figure 3: Growth of *R. palustris CGA009* at various concentrations of HMF.

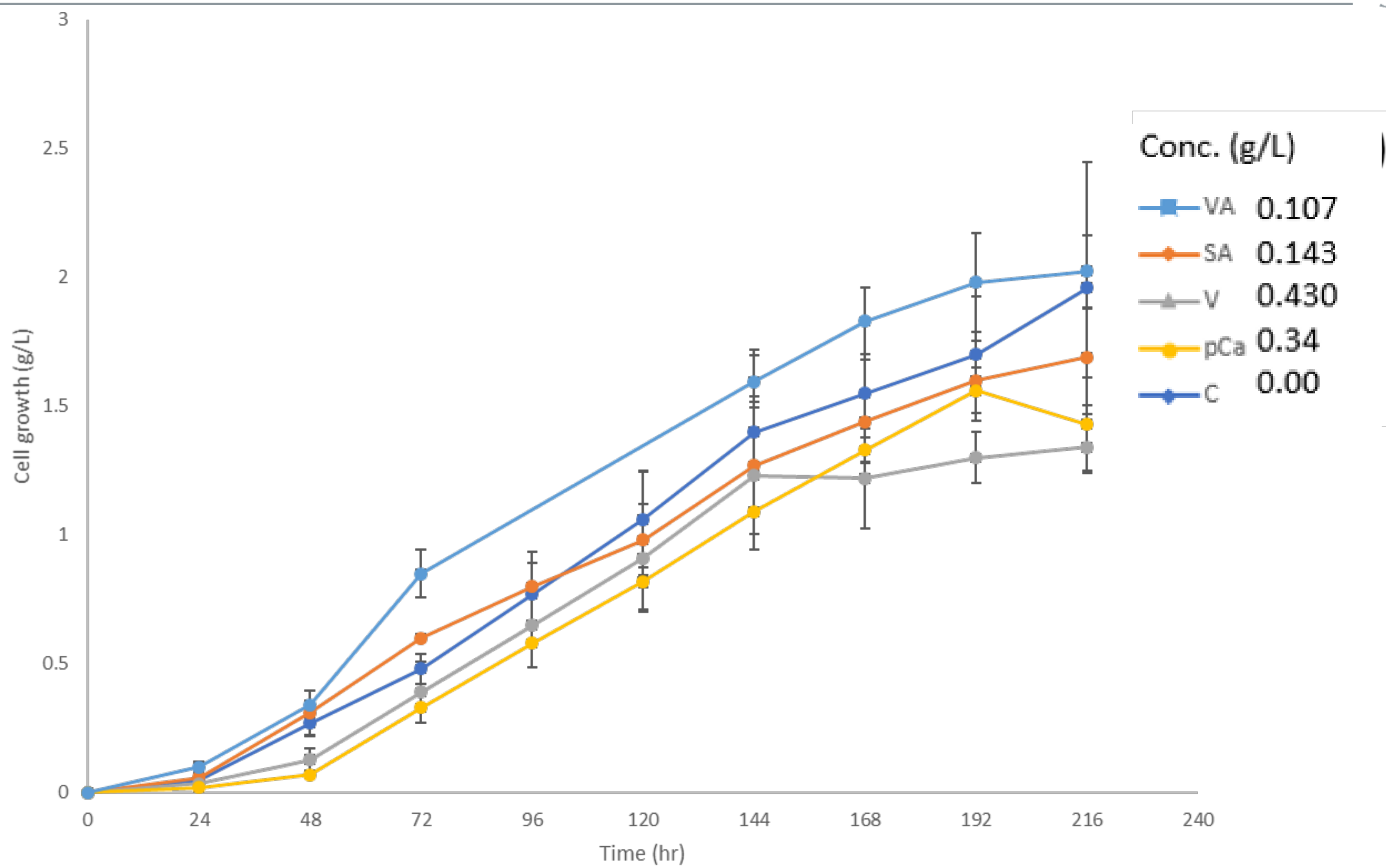

Figure 4: Cellular growth of *R. palustris* on phenolic compounds at maximum reported concentrations on literature.

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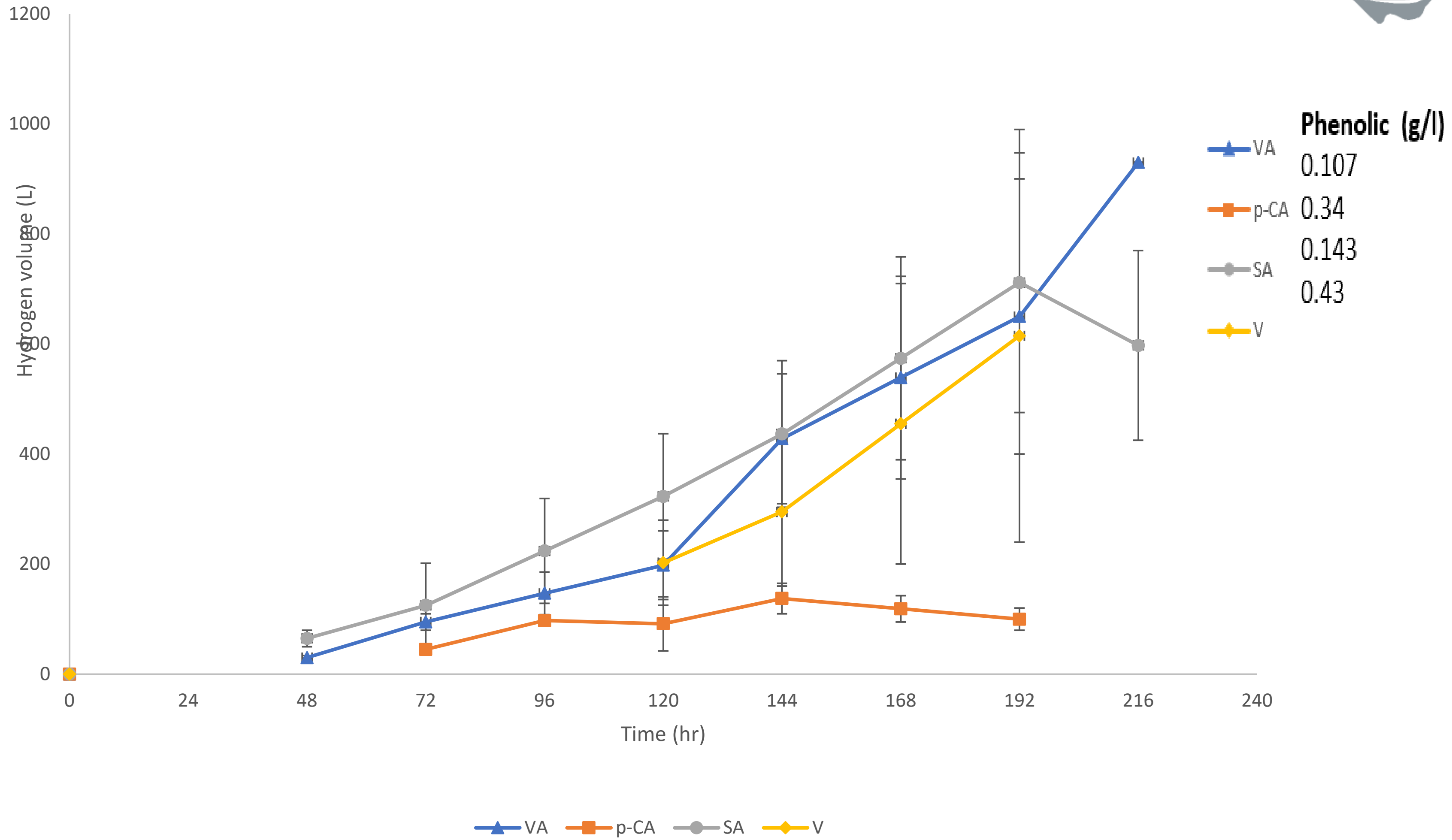

Figure 5: Cumulative hydrogen production of *R. palustris* CGA009 at various concentrations of phenolic compounds

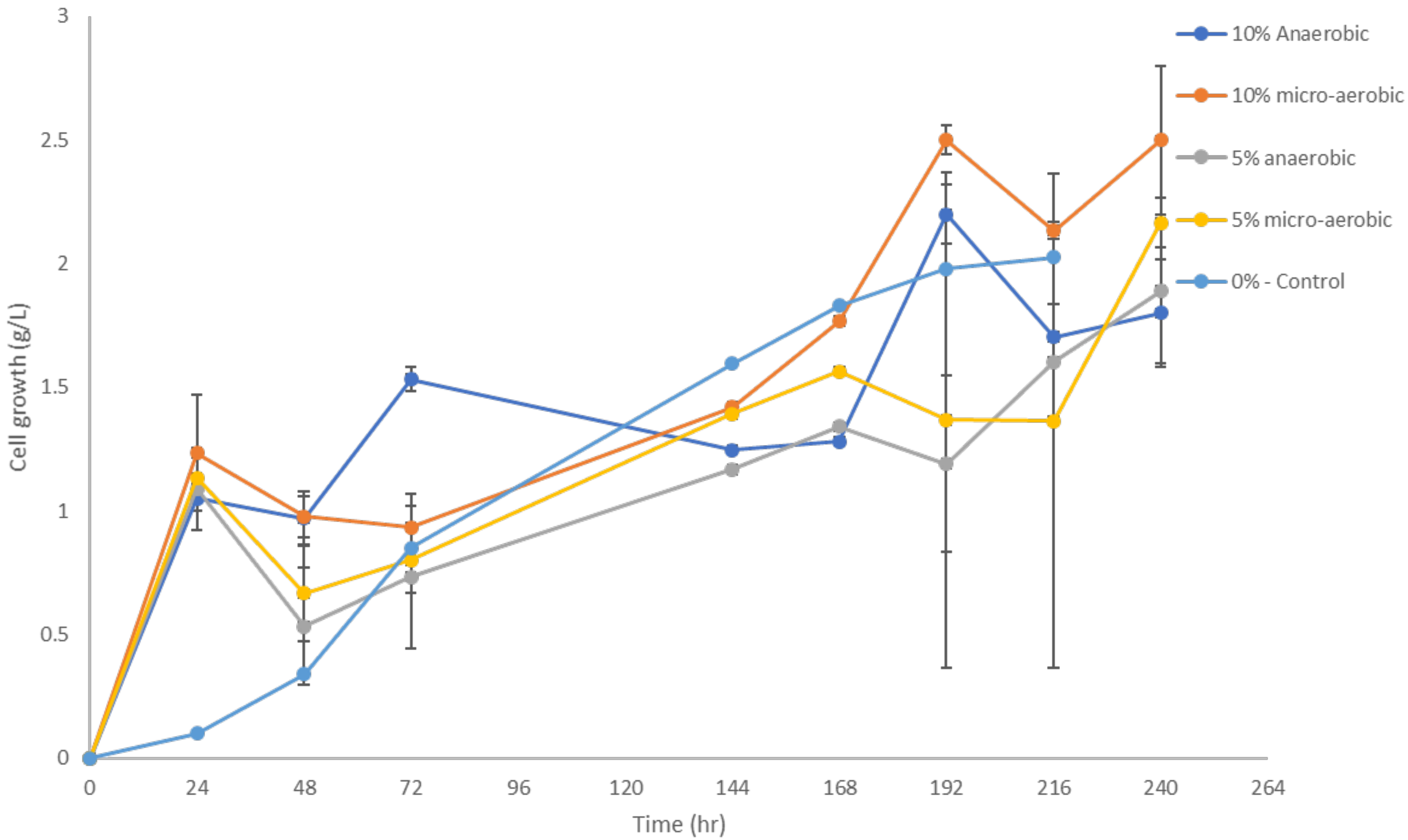

Figure 6: Cellular growth of *R. palustris* CGA009 on L.C hydrolysate under anaerobic and micro-aerobic conditions.

Conclusions

- *R. palustris* CGA009 has maximum growth between 0.97g and 1.93g
- The bacterium can grow tolerate hydrolysate dilutions above 5 %
- Micro-aerobic conditions enhance microbial growth
- Phenolic compounds affect *R. palustris* CGA009 growth and bio-hydrogen production

Current and future experimental plans

- Investigate hydrolysate photo-fermentation at % greater than 10
- Investigate effect of sequential micro-aerobic to anaerobic conditions on hydrogen production improvement
- Use *R. palustris* CGA009 acclimated on hydrolysate as an inoculum for hydrolysate fermentation
- Culture *R. palustris* CGA009 on synthetic hydrolysate medium and use as an inoculum for hydrolysate fermentation
- Perform dark micro-aerobic fermentation

Acknowledgments

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Thank you