Gas fermentation of *Cupriavidus necator* variants at elevated pressure

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Gas Fermentation Rate – commercial viability

C1-based fuels and more valuable organics need to be produced at rates which are fast enough to justify the investment in plant and people: presently big challenge for large scale production

Reasons

- Low density of gases (~ 1/1000th of liquids)
- Low gas solubility (CH4, H_2 and O_2 ~ 1 mmole/kg water, CO_2 ~ 30 mmole/kg water)

C1 gas transfer rate is slow and limits conversion rate.

Solution

Speed up gas transfer

Gas Concentration in Broth

Mass balance around bioreactor describes changes in dissolved oxygen concentration C (in milligrams per liter) in the nutrient broth gives:

$$dC/dt = k_L a\,(C^* - C) - q_{O_2}$$

gas uptake rate (mg/L/h)

Agitation and aeration (fine bubbles)

Dissolved gas conc. at eqm with inlet gas, f(T)

Gas Transfer into Liquid

Agitation and Sparging – first step

The Mechanical design of agitator blades, speed of agitation and break up into small bubbles provides an important step in getting the gas to the bacteria/cells: expressed in terms of k_La

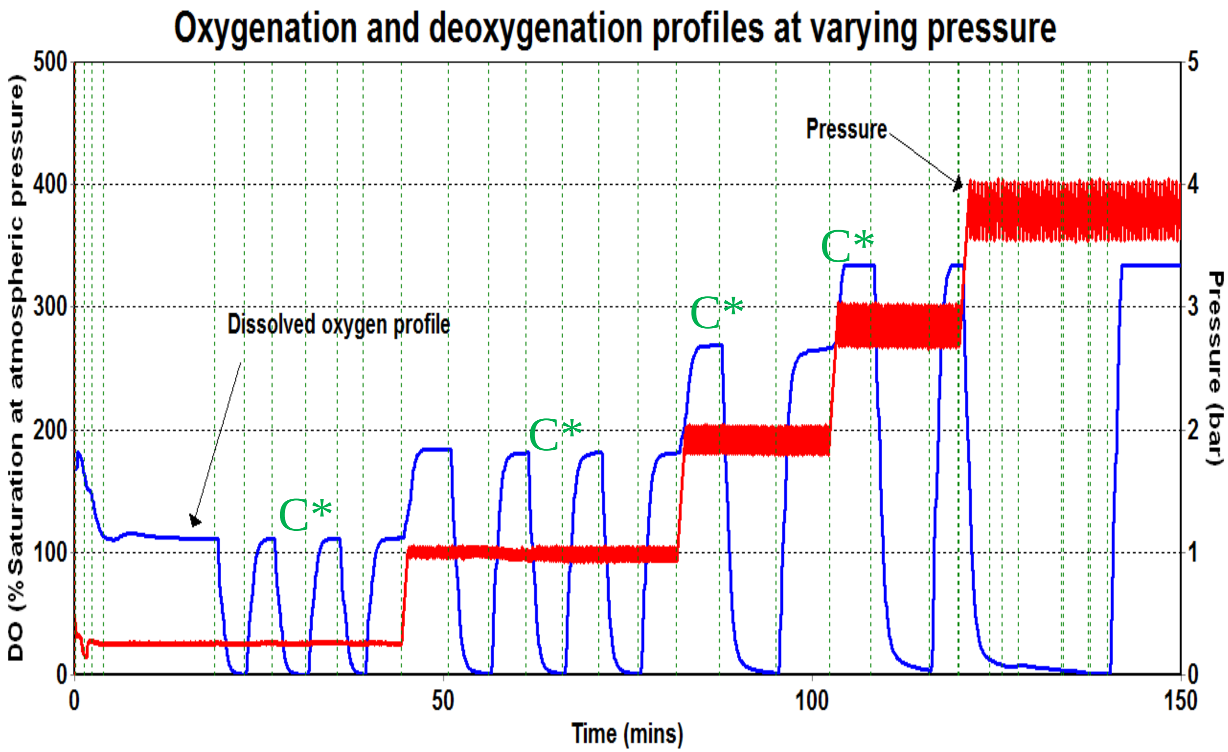
Solubility – second step

Molecular forces between the gas and liquid, expressed in terms of solubility, then determines how much gas is actually retained in the liquid.

Hence: Gas uptake rate = $k_La\,(C^* - C)$

How to increase solubility C^* ?

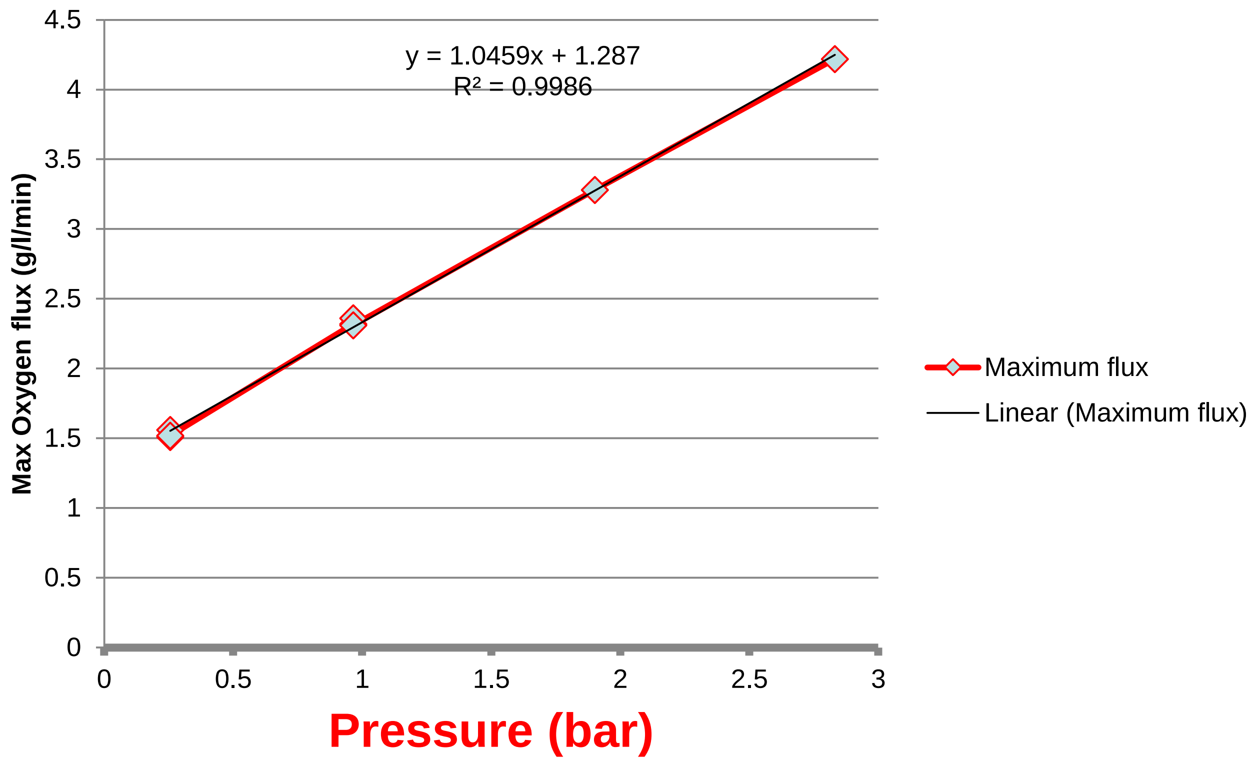
Increase Pressure - increase gas saturation level C*



Alternate oxygen and nitrogen purging at different pressure.
Oxygen saturation level increases with pressure.

Maximum Gas transfer rate into Liquid

Agitation speed, impeller and sparging fixed



Gas transfer rate increases linearly with pressure

Typical Systems used for C1 Fermentation

Fermentation pressure up to ~ 10bar

Carbon utilization of *Cupriavidus necator*

Microorganism widely recognized for conversion of C1 sources into a range of different organics, according to the choice of bacterial strain and features of bioengineering.

Three examples:

C. Necator **Re2133** (studies of **strain growth** with $CO_2/H_2/O_2$ feed)

C. Necator **pEG7d** (studies of autotropic **isopropanol production**)

C. Necator **H16** (studies of **strain growth** with both gluconate and CO_2 as carbon sources)

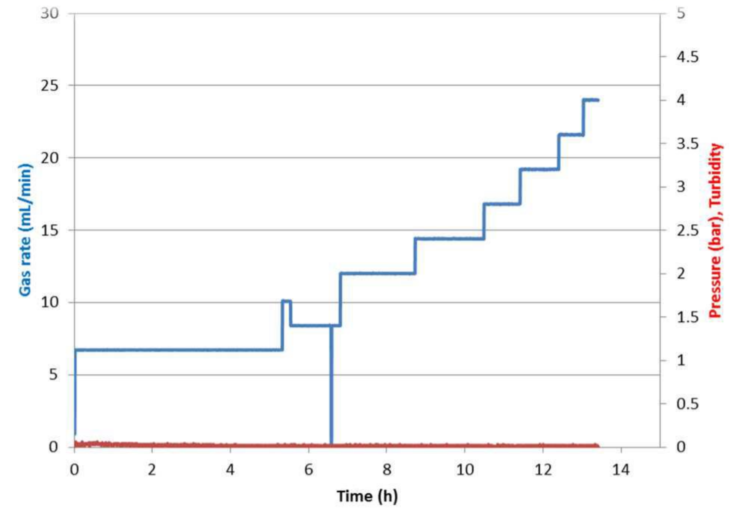
Autotrophic growth of STRAIN in bioreactor

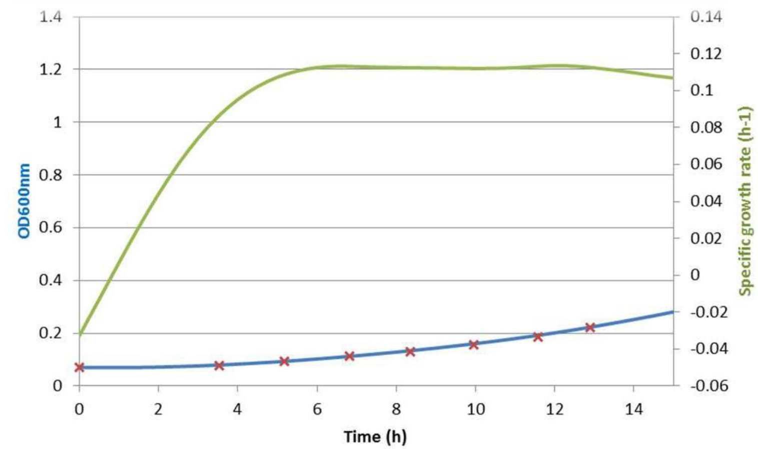
Grown in stirred reactor by supply of $CO_2/H_2/O_2$

Fermentation stopped when the maximum O_2 transfer capacity is reached (DO near zero)

maximum O_2 transfer rate determines maximum growth rate

Autotrophic growth of *Cupriavidus necator* Re2133 at atmospheric pressure





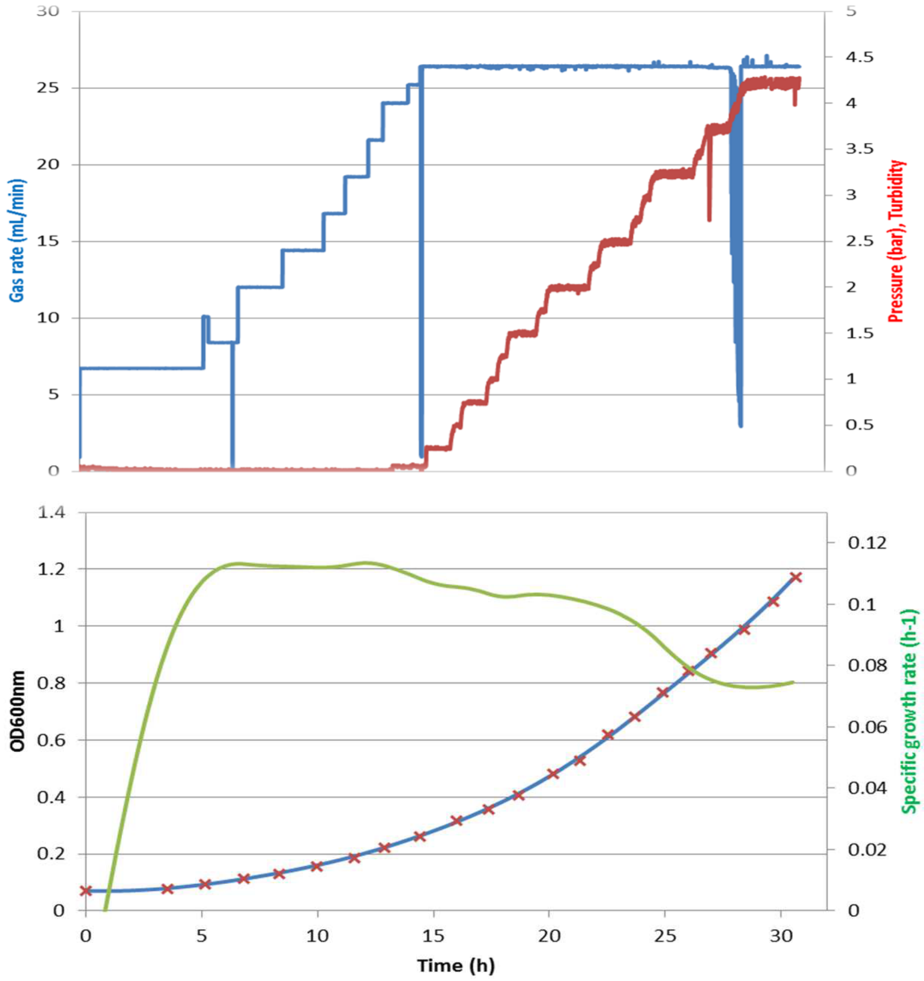
Autotrophic production of strain – at pressure

Efforts to increase growth rate by elevated pressure of gases

Pressure ramps to continue growth – up to 4 bar

Growth rate does not increase but growth is sustained for longer OD now ~ 1.2 instead of ~ 0.2 at atmospheric pressure

Autotrophic growth of *Cupriavidus necator* Re2133 at high pressures

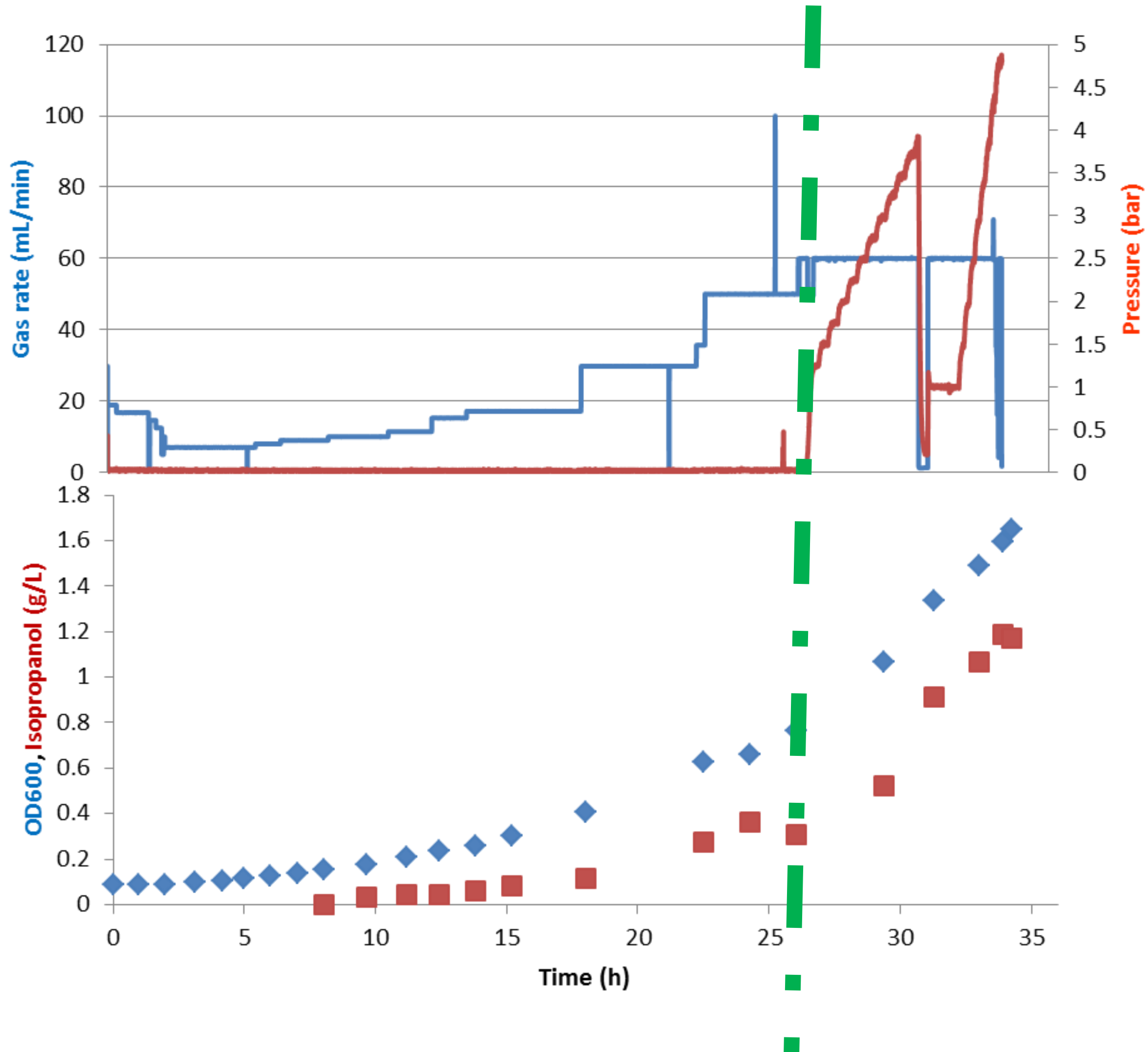


Autotrophic production of isopropanol

Autotrophic growth with Cupriavidus Necator pEG7d at high pressures

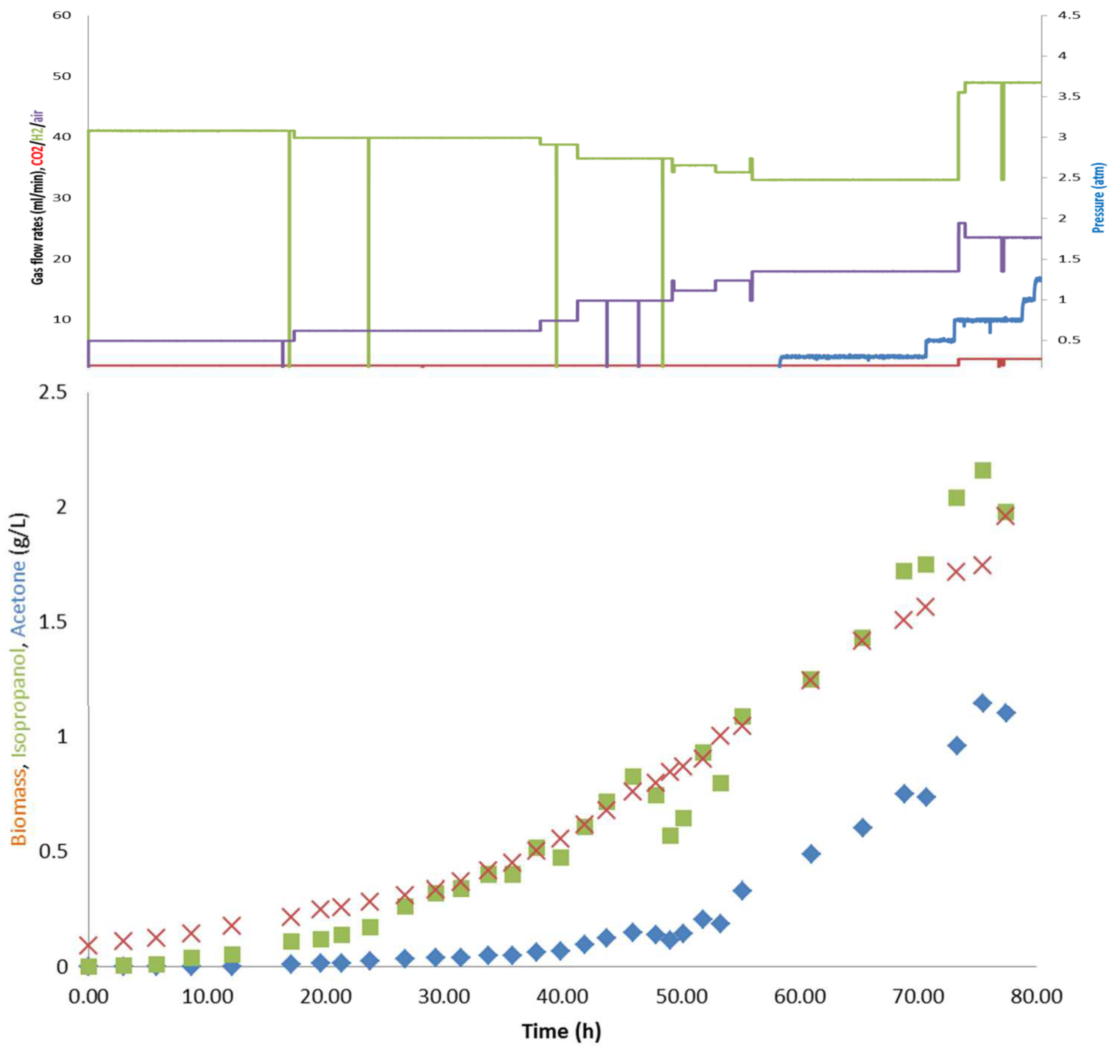
Same strategy: ramp pressure in steps when oxygen transfer limit reached

Isopropanol level of Over 1 g/L reached in 35 hours



Autotrophic production of isopropanol

Further strain Development ~ over 2 g/L isopropanol



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Isopropanol Production - benefits of elevated pressure

- **Maximum rate of IPA production in CO_2 matched rate with fructose**

Production phase on	$[isop]_{max}$ ($g\ L^{-1}$)	$qIsop_{max}$ ($Cmol\ Cmol^{-1}\ h^{-1}$)	Specific production ($g\ isop/g_{CDW}$)
CO_2 from Gas Mix	0.25	0,035	0.25
CO_2 from Ind. Gas	2.2 (3)	**0.07**	1.0
Fructose	9.0 (16)	0,062	2

Fermentation of *Cupriavidus necator* H16

Objective

C. necator **H16 during fermentation:**

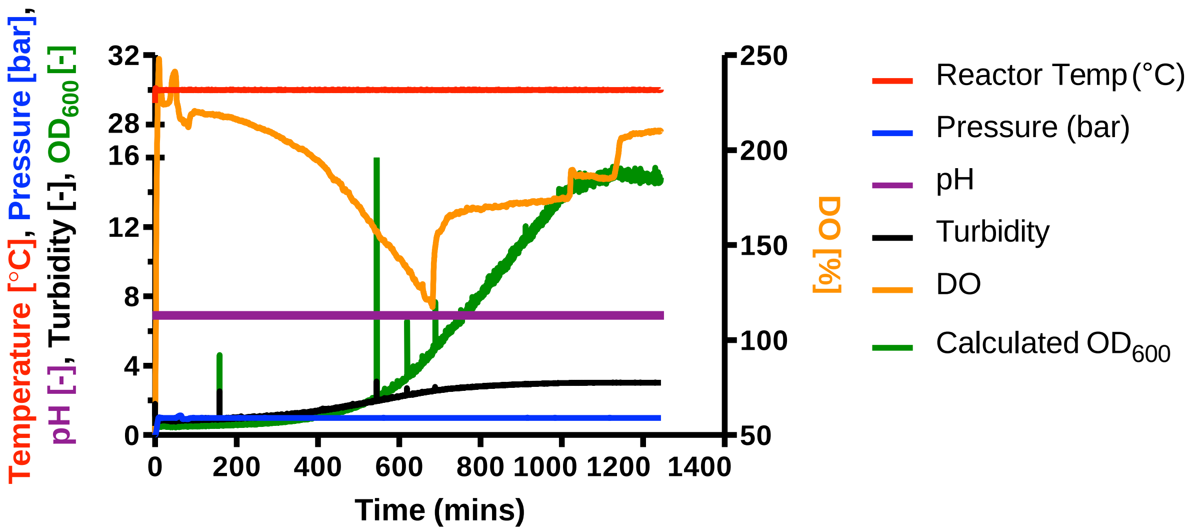
(a) under heterotrophic cultivation conditions
(b) under autotrophic cultivation conditions

Typical gas feed composition:

85 % H_2, 5 % O_2, 10 % CO_2

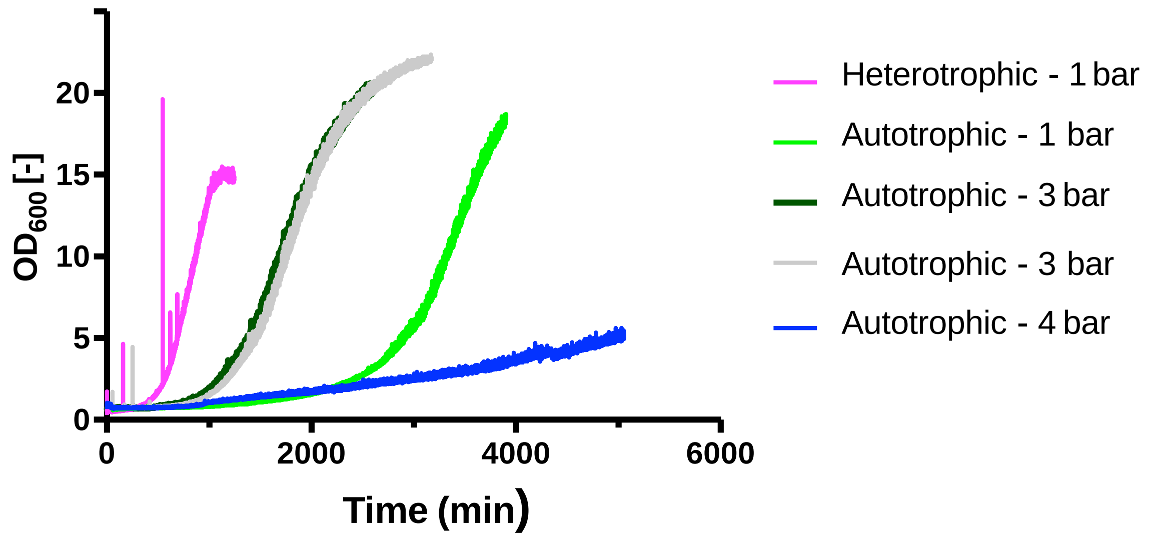
Heterotrophic growth of *C. necator* H16 at 1 bar in 1% sodium gluconate

Reference Run at 1bar



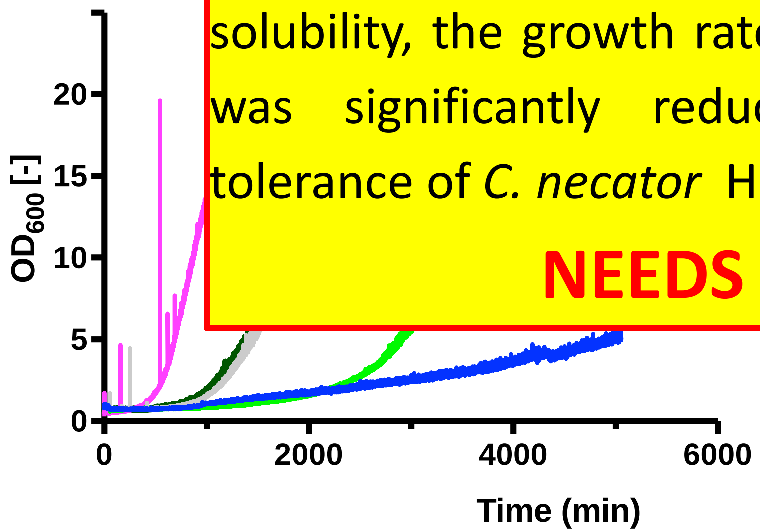
Heterotrophic growth of *C. necator* H16 at 1 bar in 1% sodium gluconate. The maximum OD_{600} (=15) and calculated μ_{max} (=0.326 h^{-1}) are similar to values achieved in tube cultures.

Heterotrophic and Autotrophic cultivation of *C. necator* H16



Growth curve of *C. necator* H16 in gas fermentation

Fermentation at 1-4 bar pressure



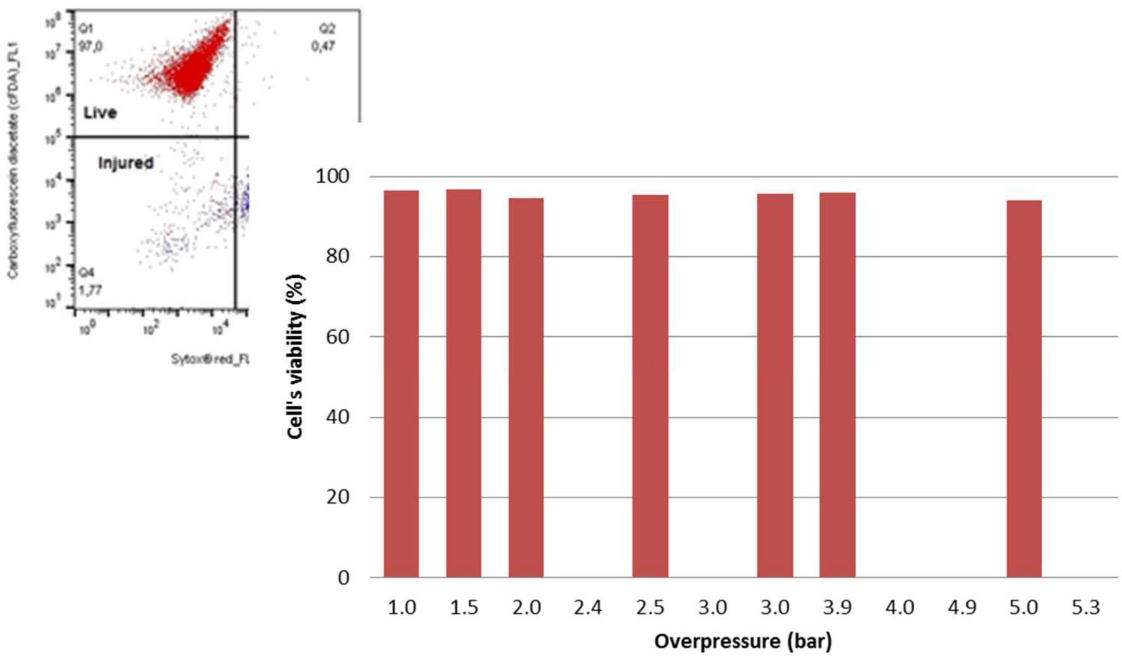
While increasing pressure to 4 bar should increase gas solubility, the growth rate of *C. necator* H16 at 4 bar was significantly reduced, suggesting the poor tolerance of *C. necator* H16 cell growth at 4 bar.

NEEDS FURTHER STUDY

Growth curve of *C. necator* H16 in gas fermentation.

Cupriavidus Necator pEG7d at elevated pressure

Cell's viability determination by Flow Cytometry



Increase in Pressure has no negative impact on cell's viability (within the range 0 to 5.3 bars overpressure)

CONCLUSIONS

1. Limits of gas solubility and density can be overcome by operation of reactor at elevated pressure.

2. Bioreactor systems operating up to 10bar have been developed and successfully operated.

3. Specific Bacterial Strains need to developed to survive high pressure conditions.

4 Cupriavidus Necator pEG7d viable at least to 5bar (GUILLOUET, LISBP, INSA, France). **Already able to produce alcohols from C1 gas to over 2g/L**

4 Cupriavidus Necator H16 at present ~ 3bar, under development (Wong and Tee at Sheffield Univ., UK).