

# **Cost effective process in lactate production with *Schizosaccharomyces pombe***

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# Agenda

## To produce lactate with lower cost

### 1. develop a strain

1. Strategy of genetic engineering of *S.pombe*
2. Selection of deleted strains

### 2. Fix a condition

1. Effect of cell density on yield
2. Batch fermentation around pH2

### 3. Show a process

1. Strategy of production
2. Simplify media
3. Repeated fermentation

# Comparison

<u><i>L.bulgaicus</i></u>	<u><i>C.glutamicum</i></u>	<u><i>S.cerevisiae</i></u>	<u><i>C.utilis</i></u>
Rate: 85 g/lh	Rate: 18 g/lh	Rate: 9 g/lh	Rate: 3 g/lh
Yield: 99 %	Yield: 66 %	Yield: 92 %	Yield: 95 %
<b>pH: 5.0</b>	<b>pH: 7.0</b>	<b>CaCO<sub>3</sub>: 35 g/l</b>	<b>CaCO<sub>3</sub>: 45 g/l</b>
(Mehaia <i>et al.</i> 1985)	(Okino <i>et al.</i> 2005)	(Saitoh <i>et al.</i> 2006)	(Ikushima <i>et al.</i> 2009)

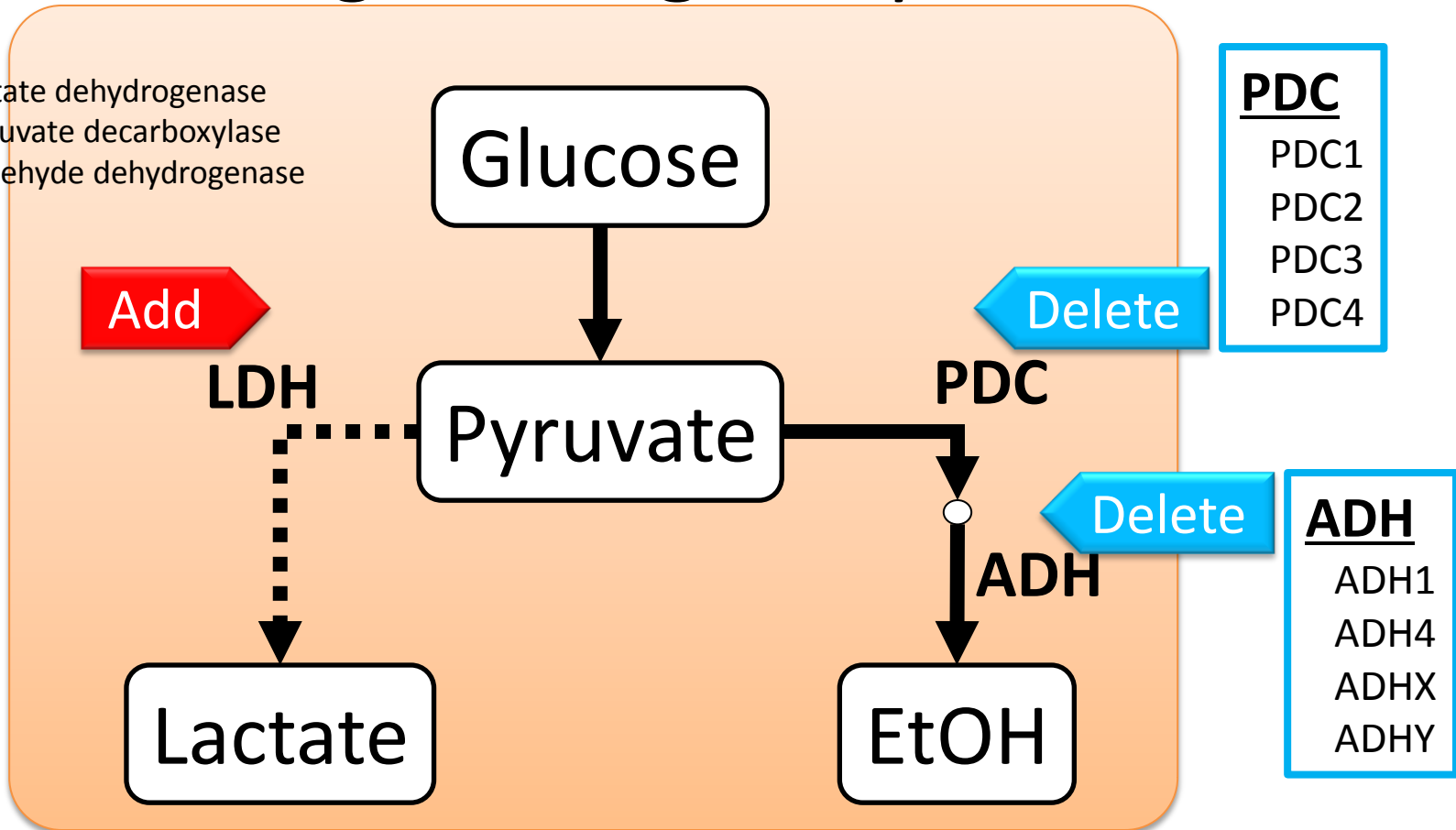
## Our goal:

Rate: 5g/lh, Yield: 80%, No-neutralize

- Neutralization cost is high
- *S.pombe* has acid tolerance
- The fission yeast produces only ethanol

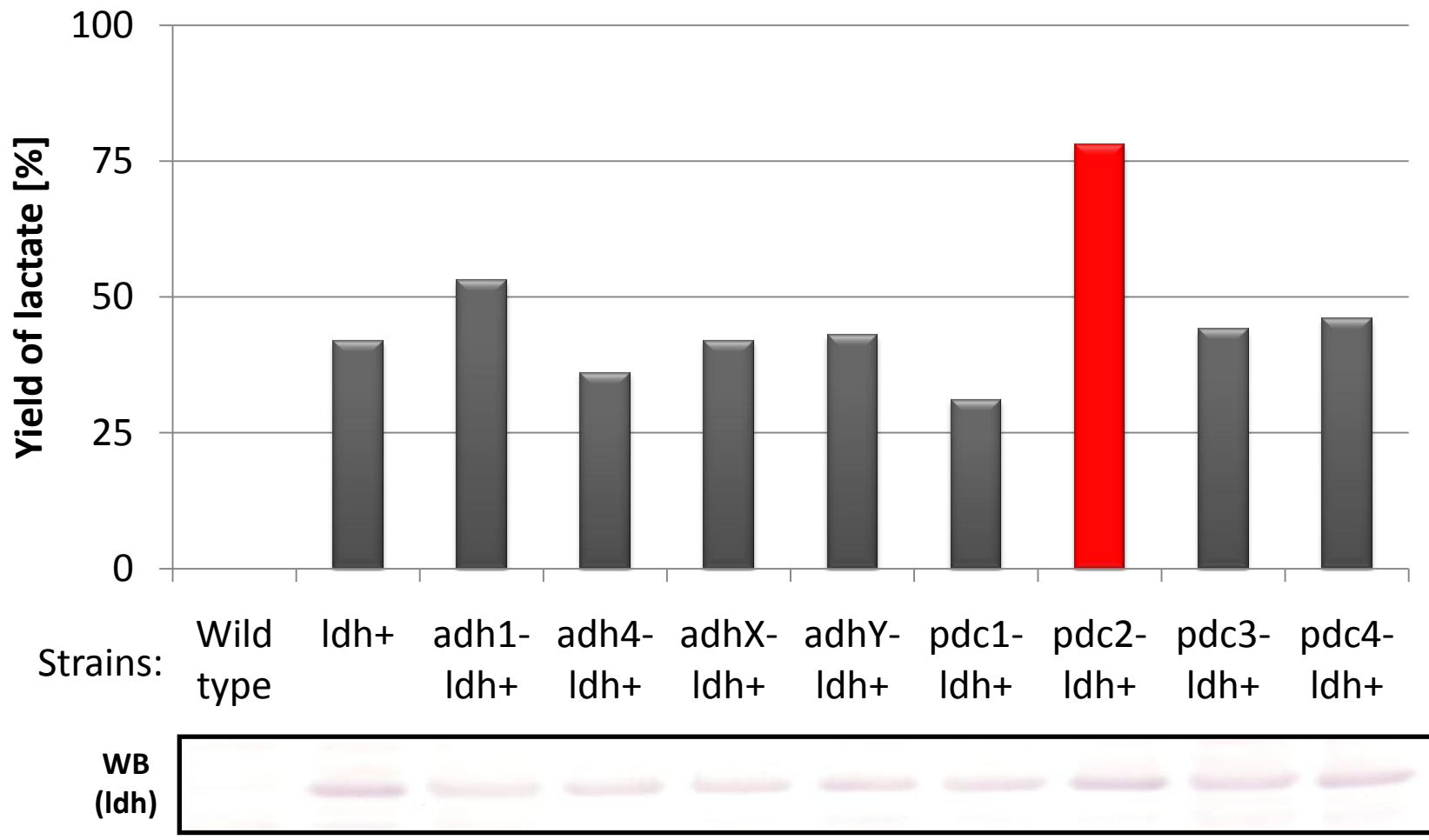
# 1-1. Strategy of genetic engineering of *S.pombe*

LDH: lactate dehydrogenase  
 PDC: pyruvate decarboxylase  
 ADH: aldehyde dehydrogenase



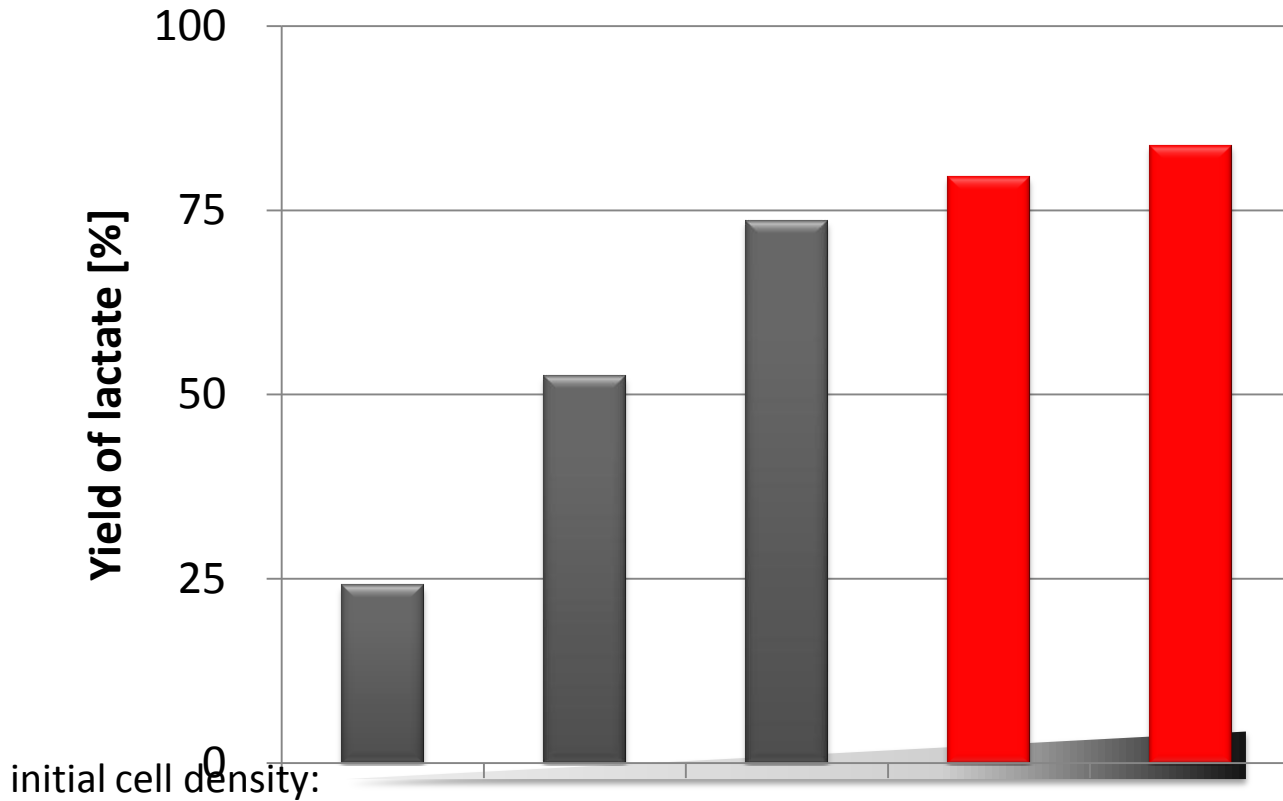
Our strategy was Added LDH, then deleted pdc or adh.

## 1-2. Selection of deleted strains



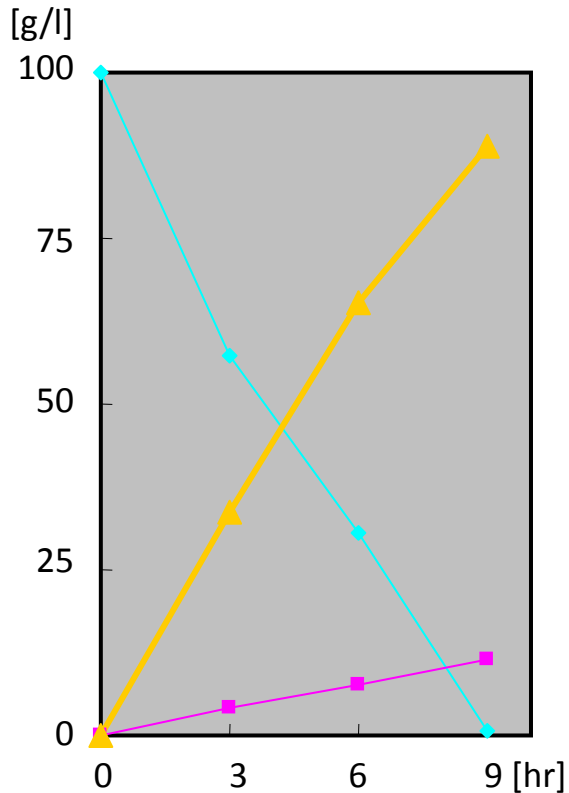
We selected the strain (pdc2-, Idh+).

# 2-1. Effect of cell density on yield

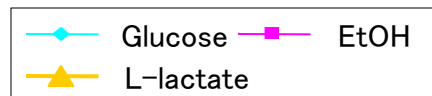


Ethanol production rate doesn't increase with initial cell density in the strain pdc2-.

## 2-2. Batch fermentation around pH2



Glucose	0.6g/l
EtOH	11.6g/l
L-lactate	88.8g/l
D-lactate	<0.2g/l
Glycerol	1.3g/l
Acetate	0.6g/l
Succinate	<0.2g/l
L-malate	<0.2g/l
Pyruvate	<0.2g/l



### Strain:

L-ldh+, pdc2-

### Media:

111g/l Glucose

### Productivity:

10g/lh

### Yield:

80%

### Optical purity:

>99.5%ee

### pH:

2.1

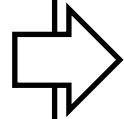
Initial cell density is 36g-dcw/l.

Fermentation of glucose solution with *S.pombe* can be

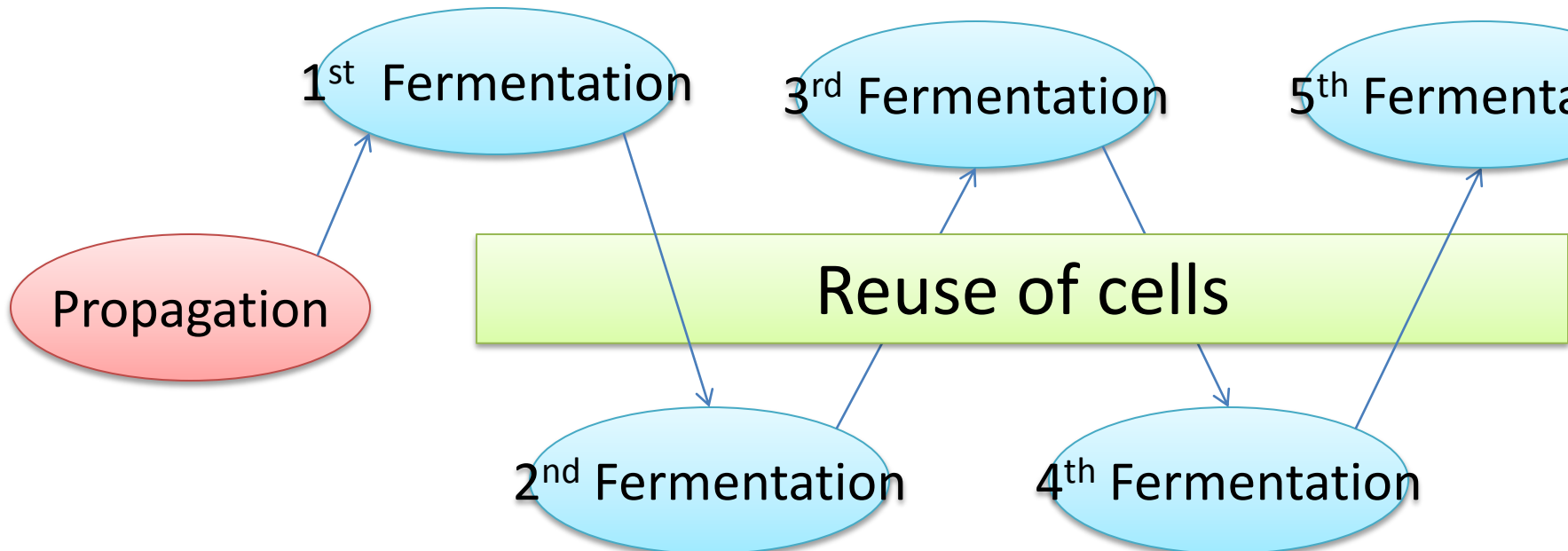
## 3-1. Strategy of production

### Characteristics of *S.pombe*

- Acid tolerance
- Robust cell wall
- Slow propagation rate



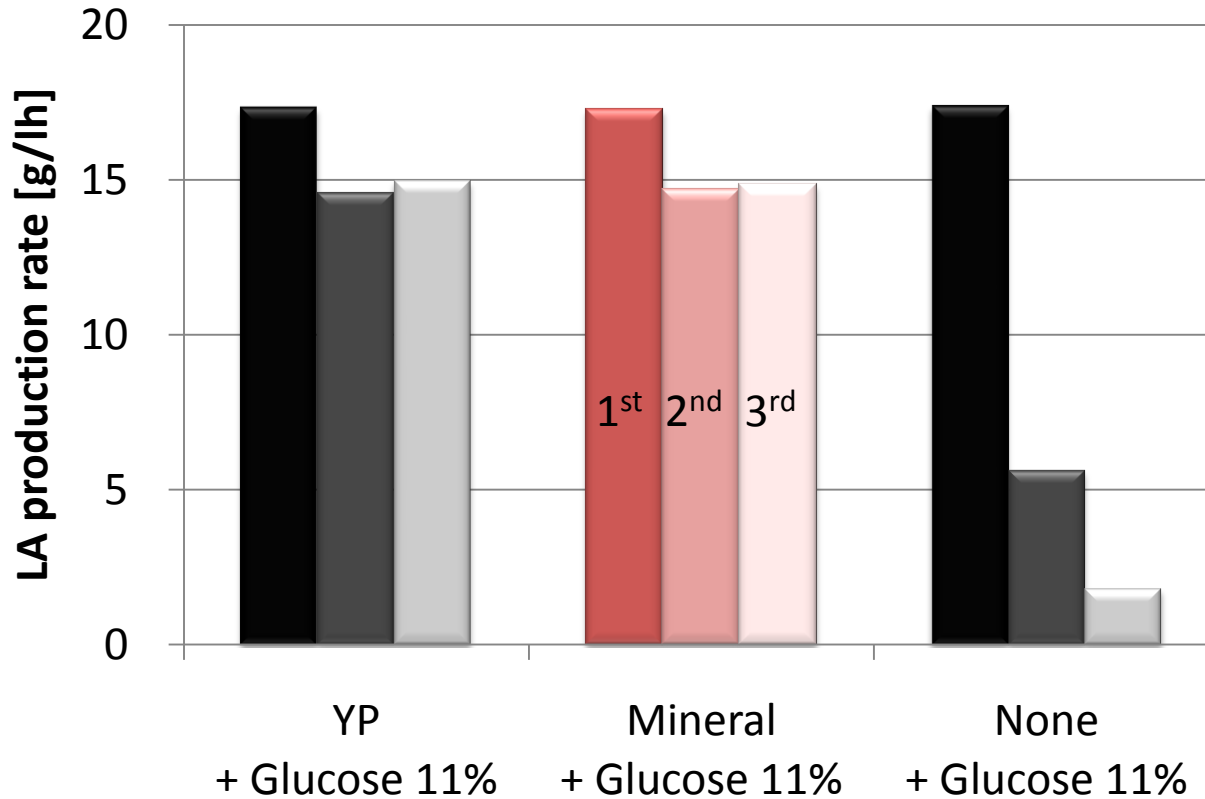
### Repeated fermentation



Repeated fermentation is better than batch fermentation for *S.pombe*.

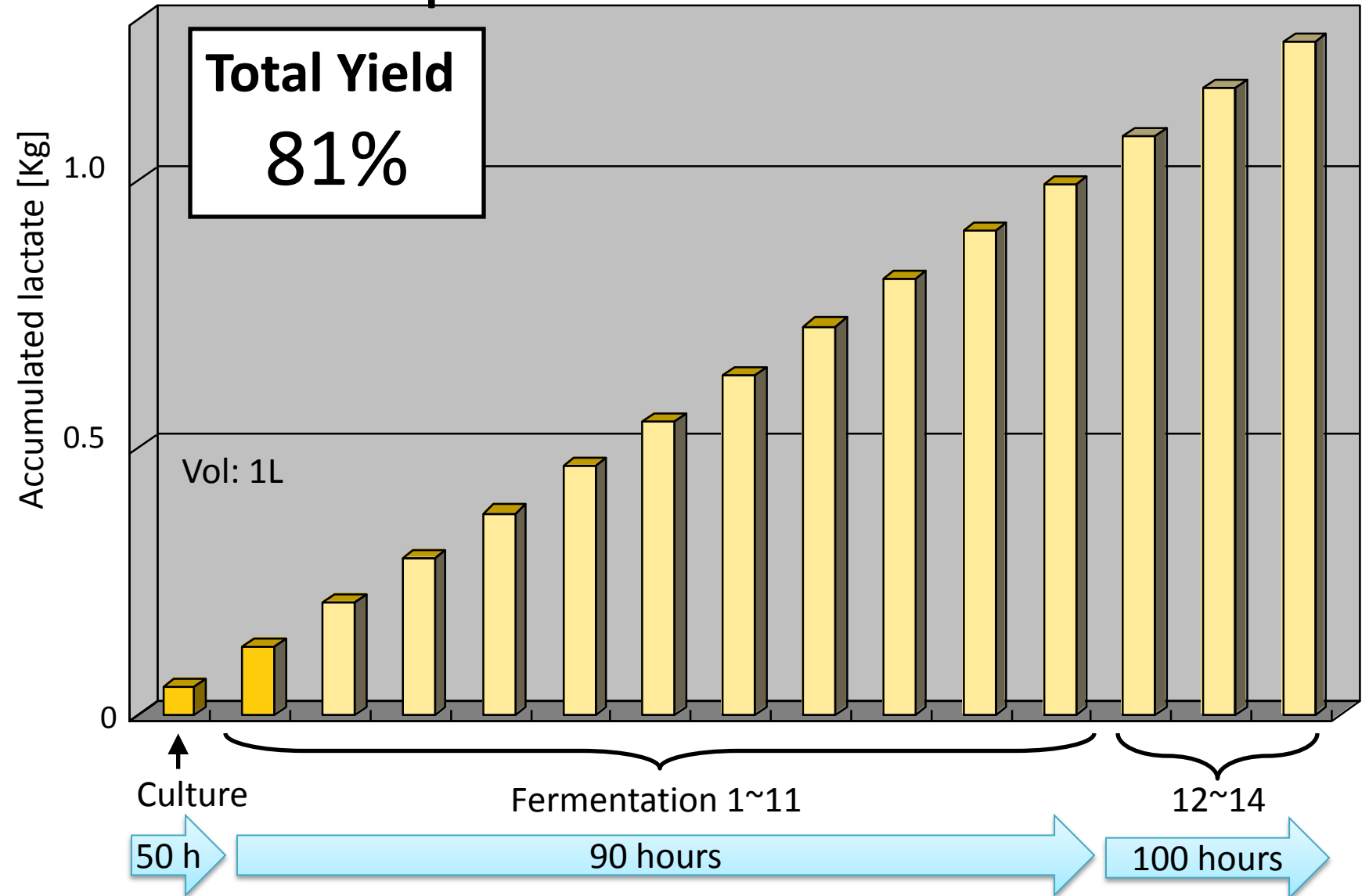


# 3-2. Simplify Media



*S.pombe* needs few amount of minerals during repeated fermentation.

# Repeated fermentation



**11 times repeating was better for production rate and yield.**

# Conclusion

- Obtain of pdc2 deleted strain
- High number of repeated fermentation
- High lactate productivity (8g/lh, 81%) at pH2.1

Our process will solve the problem of neutralization.

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Yield: 99 %	Yield: 66 %	Yield: 81 %	Yield: 92 %	Yield: 95 %
<b>pH: 5.0</b>	<b>pH: 7.0</b>	<b>pH: 2.1</b>	<b>CaCO<sub>3</sub>: 35 g/l</b>	<b>CaCO<sub>3</sub>: 45 g/l</b>

We will have a poster  
in 11th, Oct, 2012  
17:30 ~ 19:30

Thank you !