CLIMAT AmSud

Seminario 31 de marzo de 2023
Comparación entre metodologías *in vitro* para determinar la digestibilidad y potencial metanogénico de diferentes materiales forrajeros

Usefulness of the Consecutive Batch Culture technique for testing the antimethanogenic potential of some additives

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Corporación colombiana de investigación agropecuaria

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Introduction

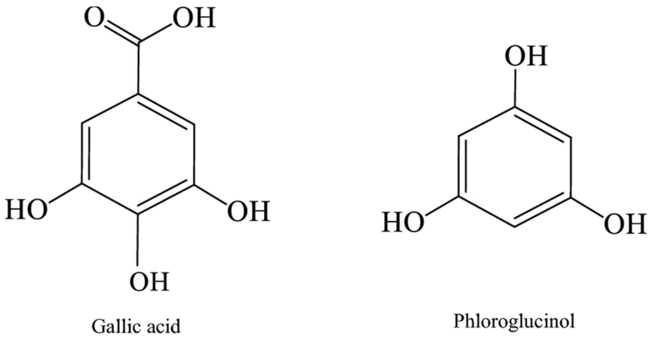
Consecutive Batch Culture

Theodorou et al http://doi.org/10.1128/aem.53.5.1046-1050.1987

- 48-h incubations
- 10% inoculum from previous batch
- Maintain fermentations characteristics similar to rumen
- Maintain fibrolytic and methanogenic functions
  - Loss of protozoa, fungi
  - Changes in prokaryote communities (possible?)
- Reported both adaptation or increased sensitivity

1)

2)



Example 1

Fungal secondary metabolites from *Monascus* spp. reduce rumen methane production in vitro and in vivo[1]

D. P. Morgavi, C. Martin, H. Boudra

AMERICAN SOCIETY OF ANIMAL SCIENCE

Journal of Animal Science, Volume 91, Issue 2, February 2013, Pages 848–860, https://doi.org/10.2527/jas.2012-5665

Why *Monascus*?

- Silage contaminant
- Secondary metabolites
  - Mycotoxins
  - HMG-CoA reductase inhibitors
    - Affect methanogens growth in culture (Miller & Wolin, 2001)
    - No effect in mixed microbes rumen culture (Busquet et al., 2005)

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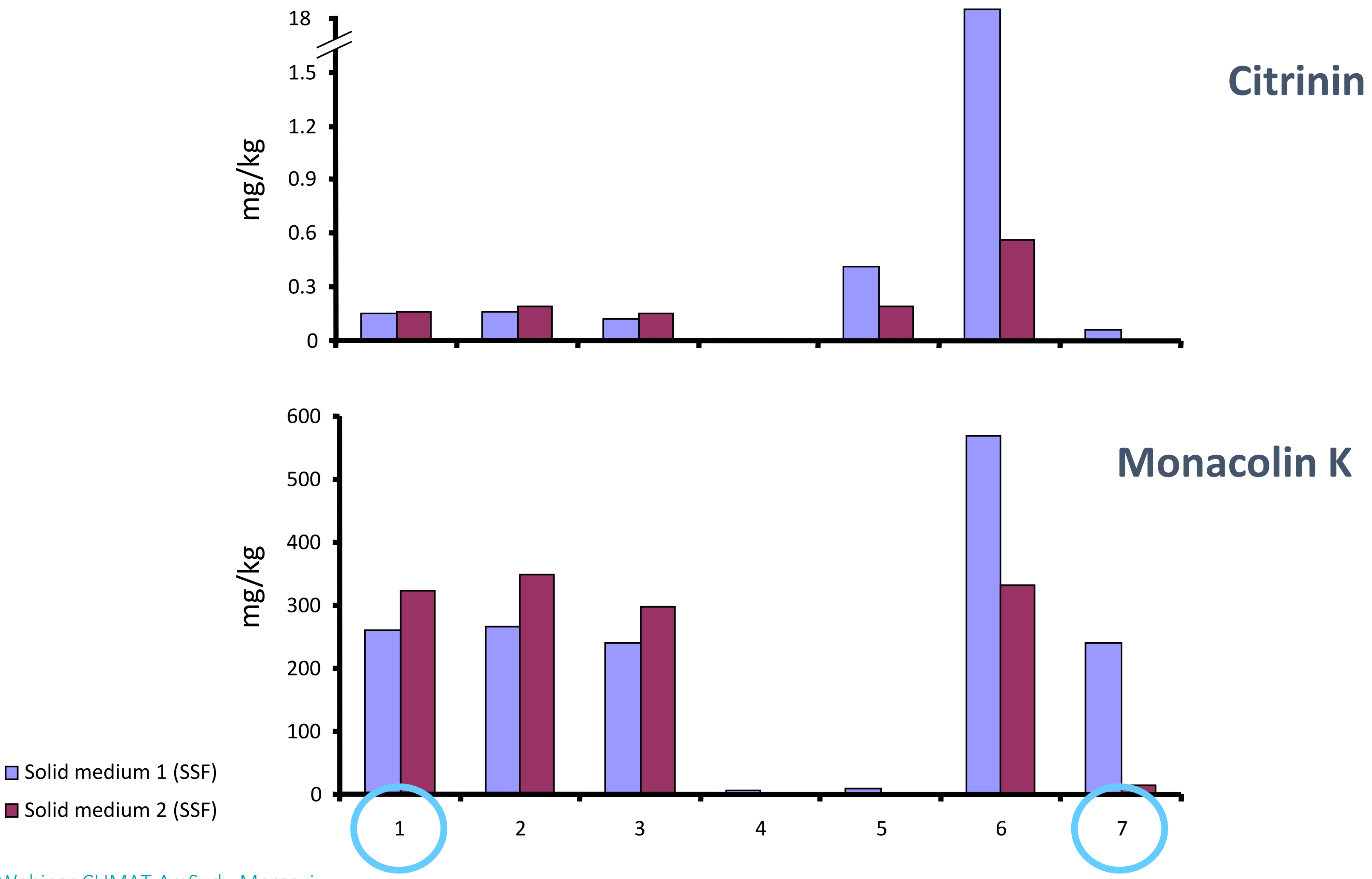
Monascus strains tested in this study

Strain	Origin	Code study
Monascus sp.	INRA URH (Corn silage)	Mon 1
Monascus sp.	INRA URH (Corn silage)	Mon 2
Monascus sp.	INRA URH (Corn silage)	Mon 3
Monascus sp.	INSA Toulouse	Mon 4
Monascus ruber	INSA Toulouse	Mon 5
Monascus purpureus	DSMZ 1603 (Chinese anka)	Mon 6
Monascus ruber	DSMZ 62748 (corn silage)	Mon 7

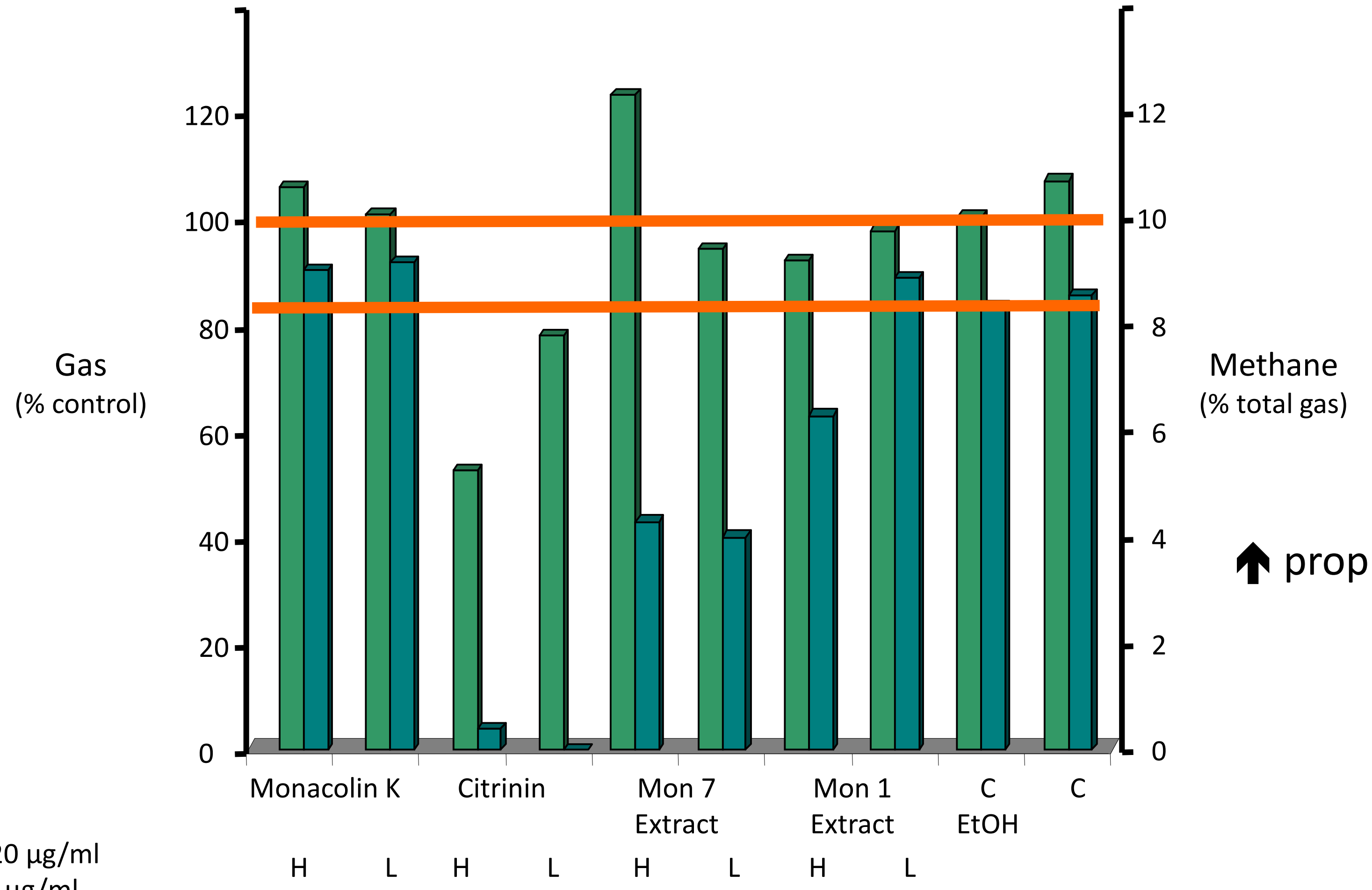
Production of secondary metabolites by *Monascus* strains



Effect of *Monascus* spp. metabolites on rumen fermentation characteristics of 18-h in vitro incubations

Treatment[1]	Gas[2] mL	Methane[2] µmol	Total VFA mmol/L	Acetate (Ac)	Propionate (Pr)	Butyrate	Iso-acids	Minor[3]	Ac:Pr ratio
Control	5.8	61.2	96.1	71.2	22.1	4.8	1.0	0.9	3.2
Monascus 1 low	5.9	64.4	98.5	70.6	22.6	5.0	0.9	0.9	3.1
Monascus 1 high	5.5	45.1	93.5	63.6*	28.1*	6.2*	0.9	1.2[4]	2.3*
Monascus 7 low	5.7	28.4*	102.1	63.2*	29.3*	5.9*	0.6*	1.0	2.2*
Monascus 7 high	7.4*	33.6[4]	111.3*	54.8*	35.3*	8.3*	0.4*	1.2	1.6*
Monacolin low	6.1	60.3	99.0	70.8	22.2	4.9	1.0	1.0	3.2
Monacolin high	6.4	67.4	100.6	72.2	21.2	4.6	1.0	0.9	3.4
Citrinin low	4.7*	ND*[5]	82.0*	63.7*	28.0*	5.9*	1.2*	1.2	2.3*
Citrinin high	3.1*	2.4*	64.4*	63.6*	28.4*	5.8[4]	1.4*	0.9	2.2*
SEM	0.19	6.59	2.99	0.76	0.70	0.27	0.04	0.10	0.08
P-value[6]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

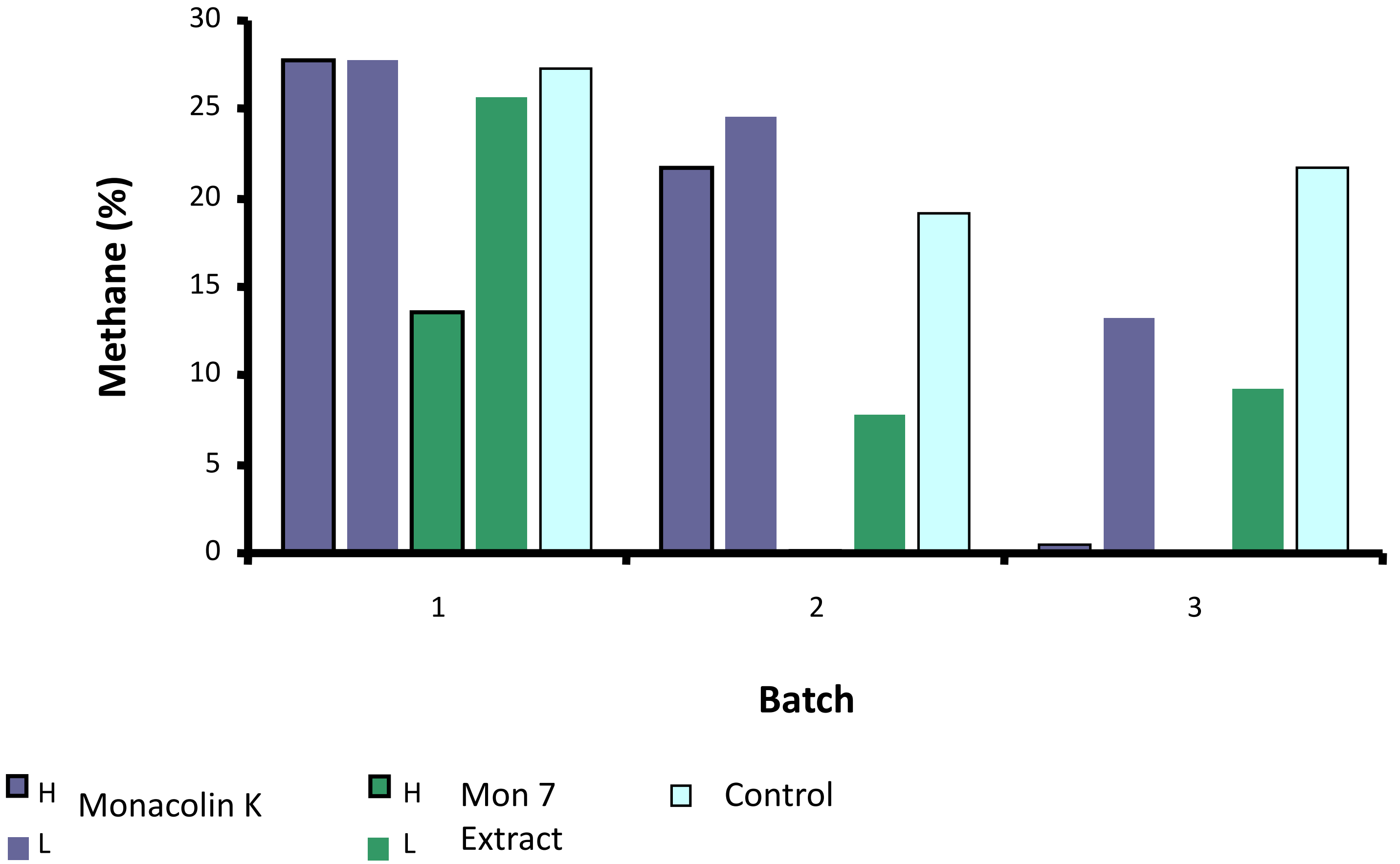
Effect of *Monascus* extracts on rumen fermentation in vitro



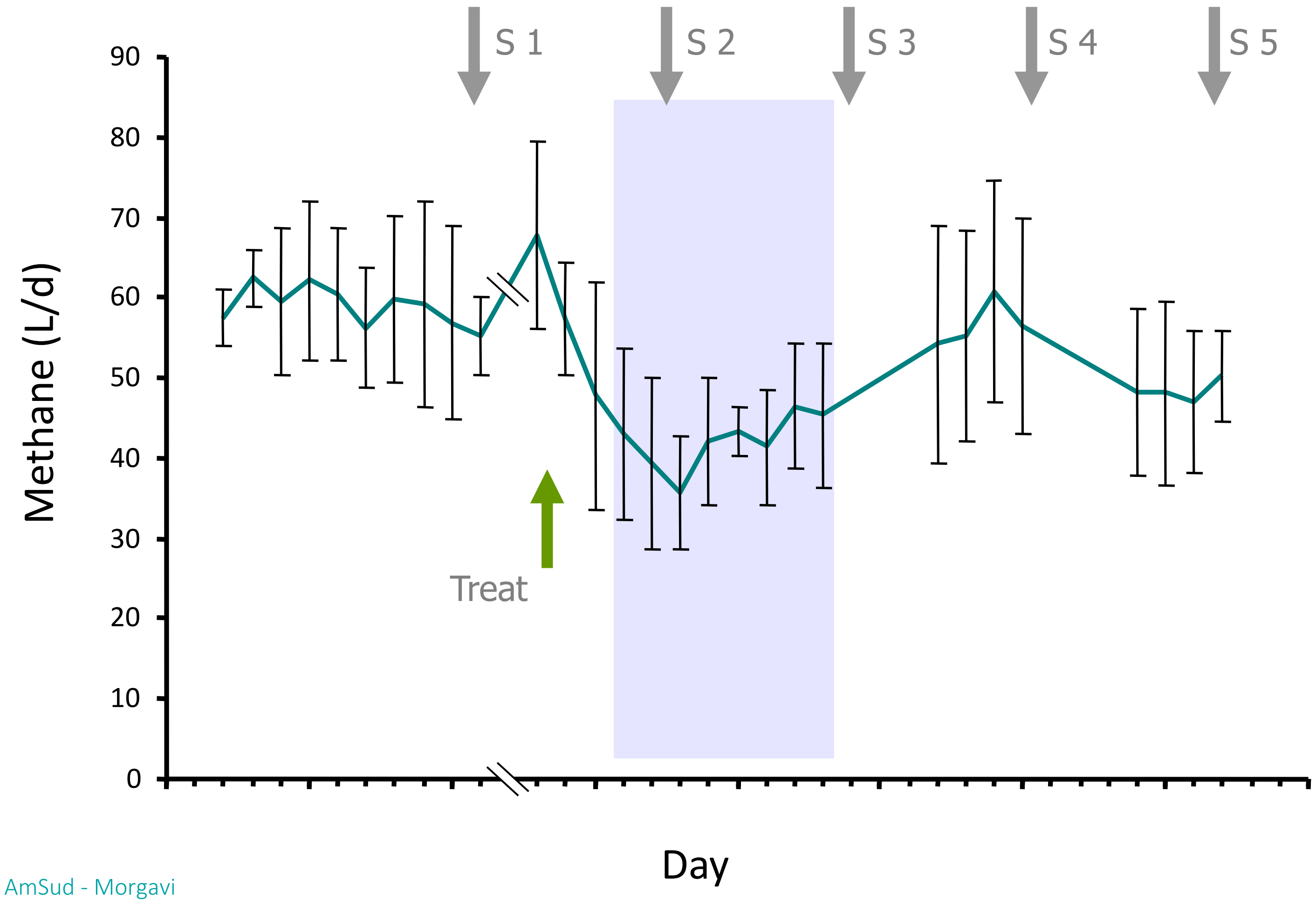
⬆ propionate

H= 20 µg/ml
L= 5 µg/ml

Consecutive batch culture



Daily Methane Production



Example 2

Evaluating the effect of phenolic compounds as hydrogen acceptors when ruminal methanogenesis is inhibited *in vitro*

Part 1 - Dairy cows Huang et al. Animal 2023

Part 2 - Dairy goats Romero et al. Animal 2023

- Rationale

$$Gallate^{-} + H_2 + 3\,H_2O \longrightarrow 2\,acetate^{-} + 0.5\,butyrate^{-} + HCO_3^{-} + 2.5\,H^{+}$$

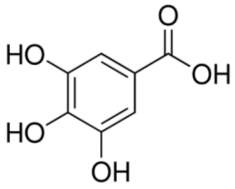
$$Pyrogallol^{-} + H_2 + 2\,H_2O \longrightarrow 2\,acetate^{-} + 0.5\,butyrate^{-} + 2.5\,H^{+}$$

$$Phloroglucinol^{-} + H_2 + 2\,H_2O \longrightarrow 2\,acetate^{-} + 0.5\,butyrate^{-} + 2.5\,H^{+}$$

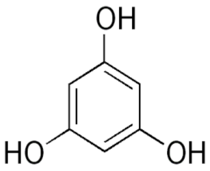
(Krumholz et al., 1986)

Hypothesis:

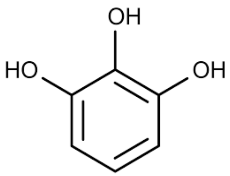
Phenolic compounds can capture excessive hydrogen in rumen ecosystem when methane production is inhibited.



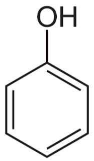
Gallic acid



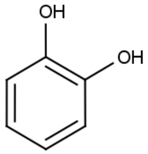
Phloroglucinol



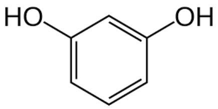
Pyrogallol



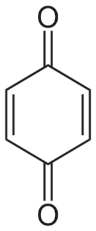
Phenol



Catechol



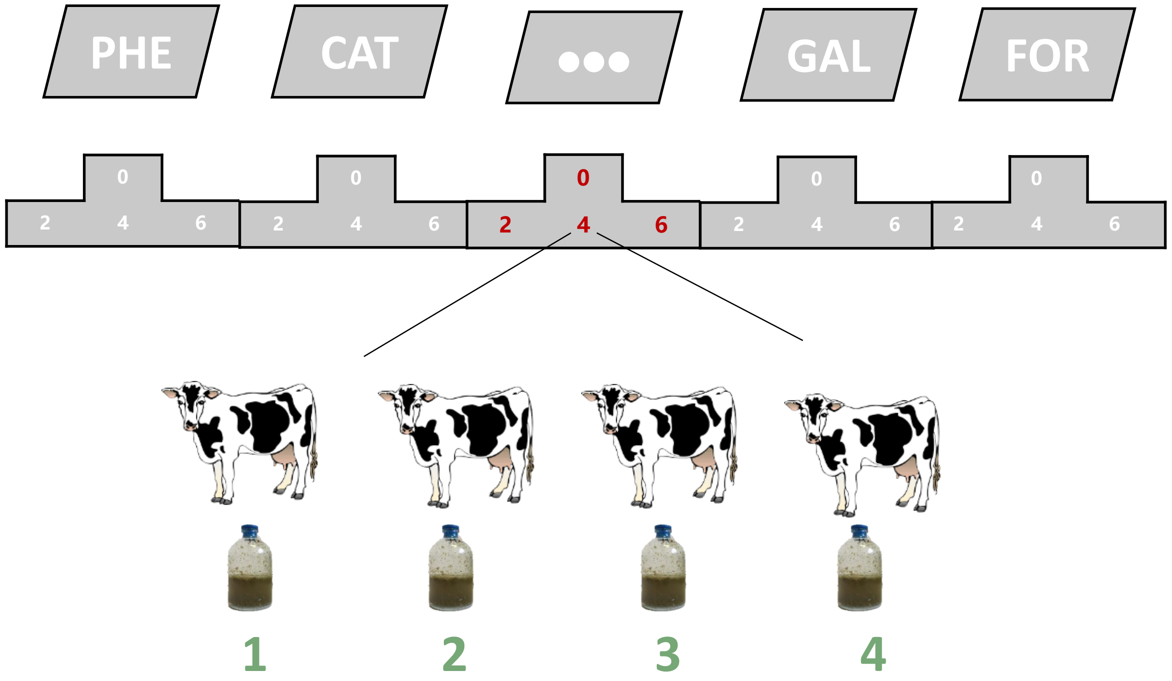
Resorcinol



Hydroquinone

From Huang 2021

Exp.1 Screening of optimum concentration of each phenolic compound



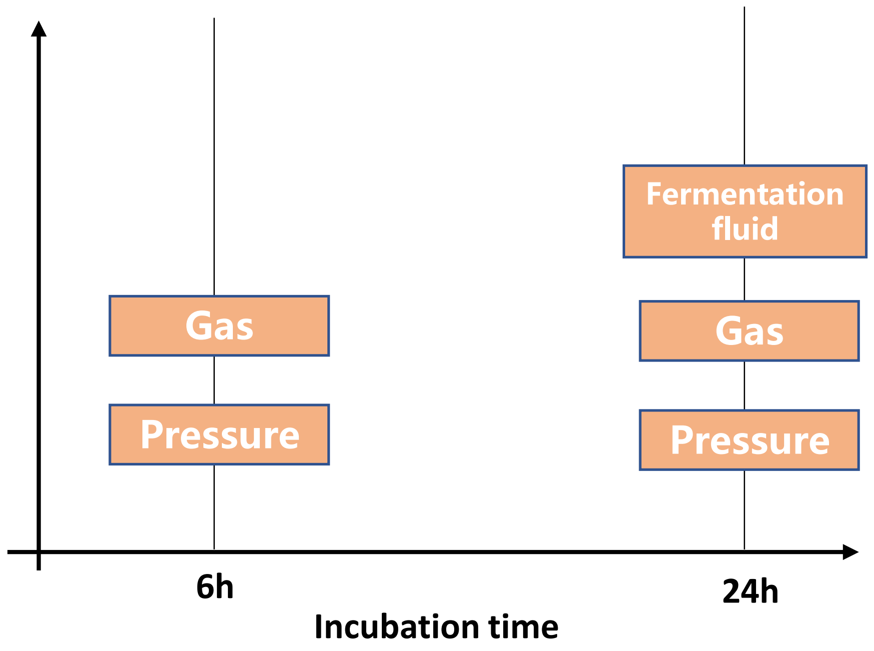
PHE: Phenol
CAT: Catechol
RES: Resorcinol
HYD: Hydroquinone
PHL: Phloroglucinol
PYR: Pyrogallol
GAL: Gallic acid
FOR: Formic acid

- **Doses: 0, 2, 4 and 6 mM**
- **Repeats: 4**
- **Incubation time: 24h**

From Huang 2021

Exp.1 Screening of optimum concentration

Sample collection

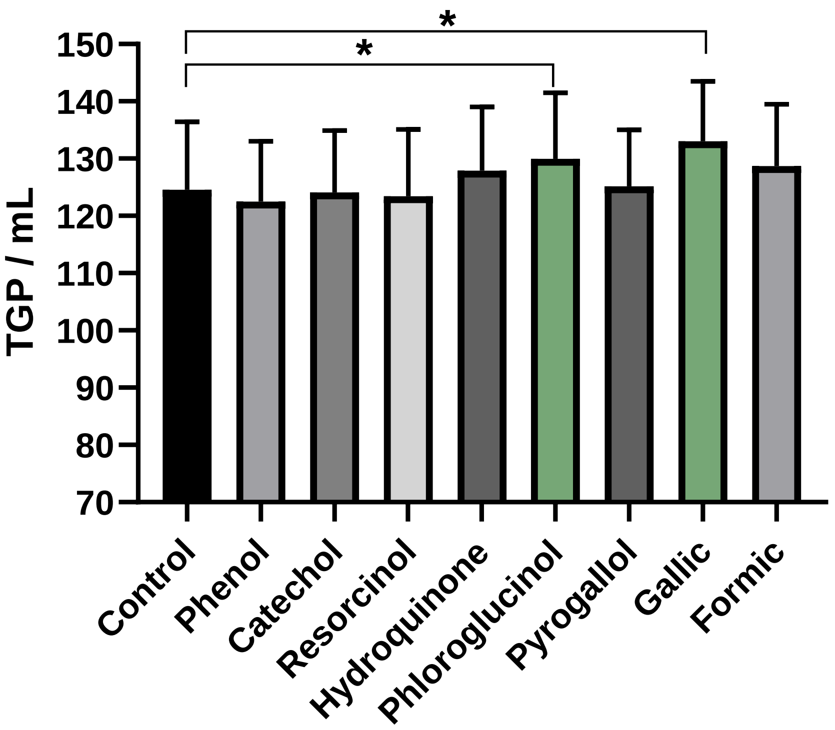


Sample	Analysis
Gas	H2, CH4, TGP
Fermentation fluid	VFA, pH, ammonia

From Huang 2021

Exp.1 Screening of optimum concentration

Results



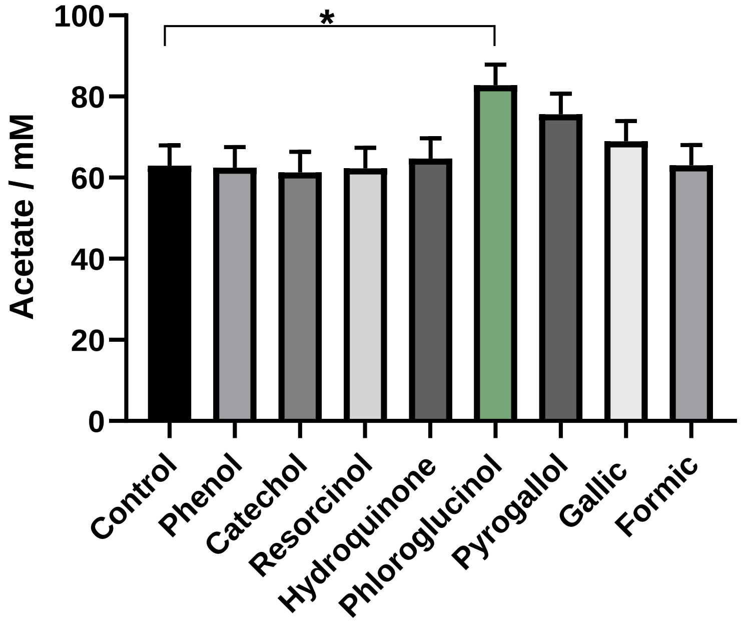
TGP during 0 - 24h fermentation when added 6 mM of each phenolic compound

- 6 mM phloroglucinol and gallic acid significantly increased TGP by 4% and 7% , respectively
- 6 mM other phenolic compounds have no negative effect on TGP

From Huang 2021

Exp.1 Screening of optimum concentration

Results



Acetate production during 0 - 24h fermentation when added 6 mM of each phenolic compound

- 6 mM phloroglucinol significantly increased acetate production by 24%
- 6 mM phenolic compounds have no negative effect on VFA production or NH3 accumulation
- 6 mM of each phenolic compound was chosen because there are no negative effect on fermentation

From Huang 2021

Exp.2 Combine optimum concentration of each phenolic compound with methane inhibitors

Methane inhibitor + **Phenolic compound** → 

- 1.5% seaweed
- 2.5% seaweed
- 3 µM BES

- 6 mM

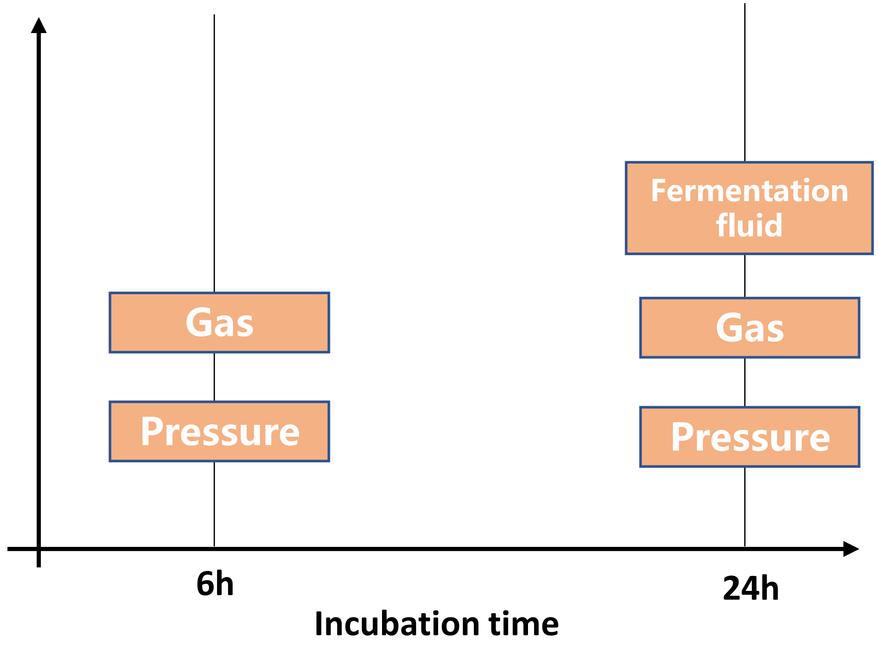
- Incubation for 24h

Different inclusion levels of methane inhibitors were considered to achieve different hydrogen accumulation

From Huang 2021

Exp.2 Combine phenolic compound with methane inhibitors

Sample collection

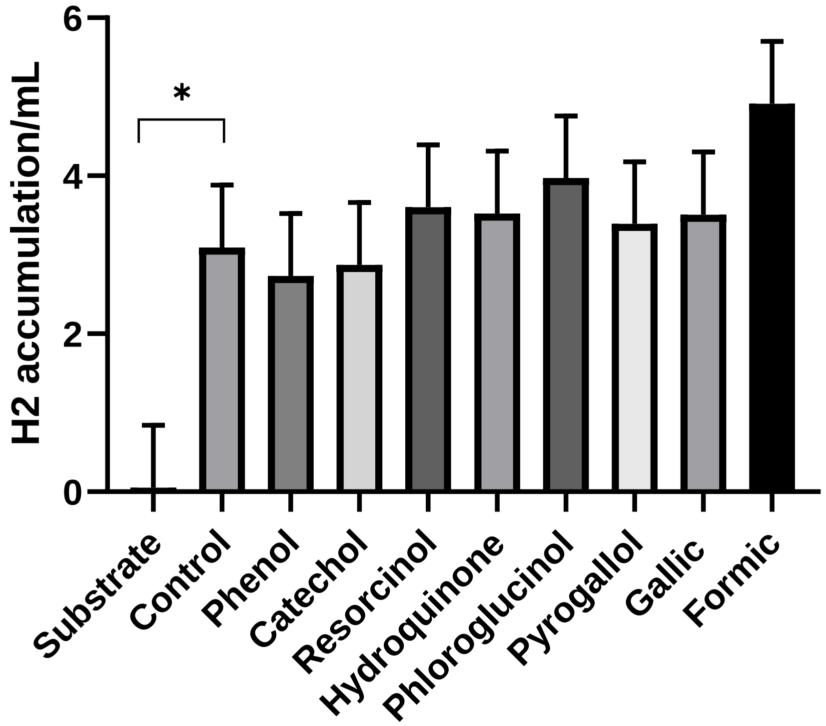


Sample	Analysis
Gas	H2, CH4, TGP
Fermentation fluid	VFA, pH, ammonia

From Huang 2021

Exp.2 Combine phenolic compound with methane inhibitors

Results



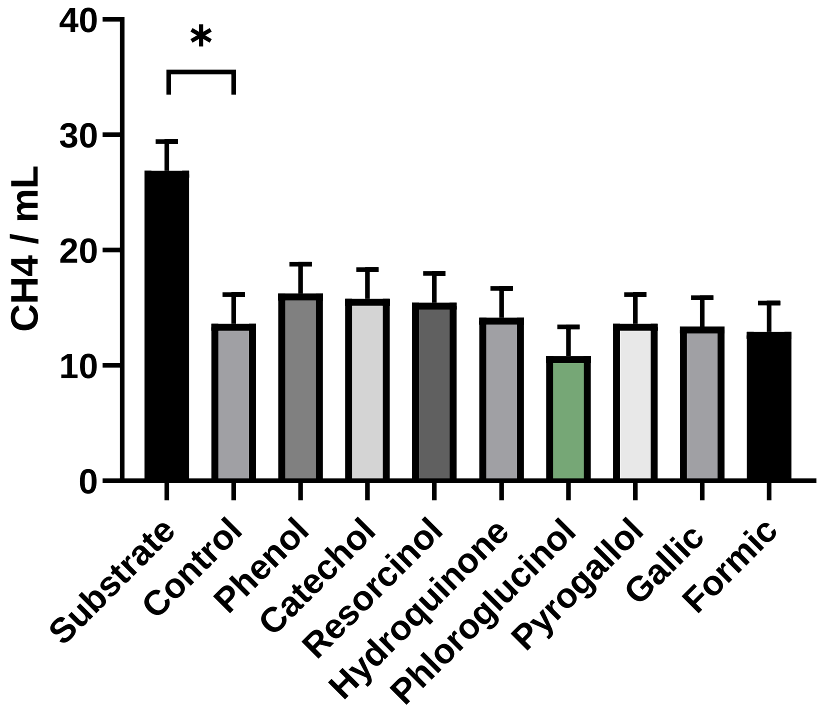
Effect of phenolic compounds on H2 accumulation when added methane inhibitors during 0 - 6h fermentation

- H2 accumulation significantly increased when added methane inhibitor compared to substrate treatment
- There were no significant effect on H2 accumulation for all the phenolic compound treatments compared to control treatment

From Huang 2021

Exp.2 Combine phenolic compound with methane inhibitors

Results



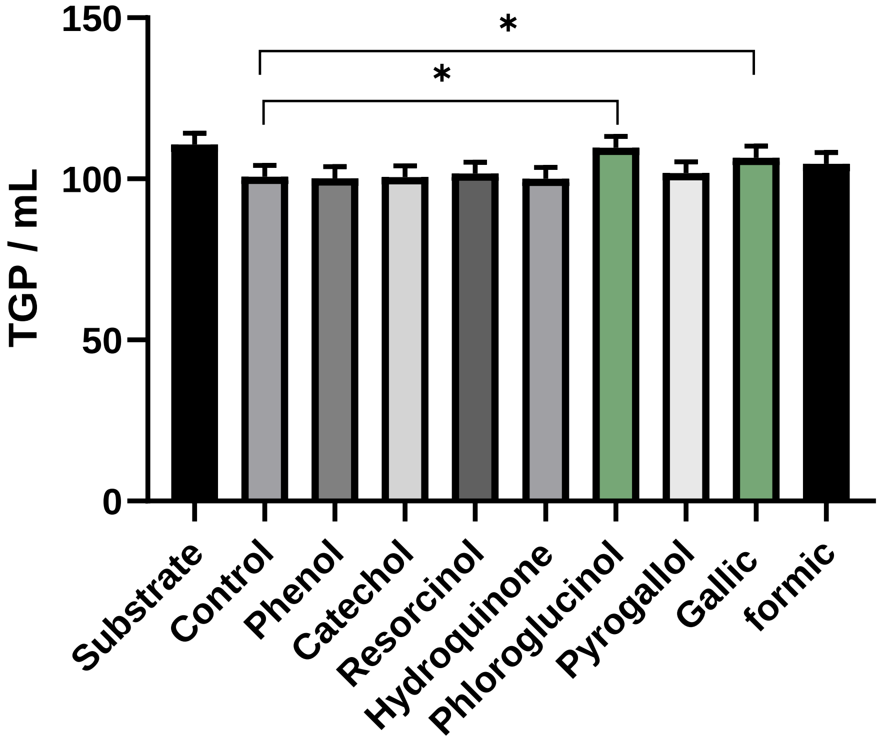
Effect of phenolic compounds on CH_4 production when added methane inhibitors during 0 - 24h fermentation / ml

- CH_4 production significantly decreased when added methane inhibitor compared to substrate treatment
- Phloroglucinol numerically decreased CH_4 production by 20% compared to control treatment

From Huang 2021

Exp.2 Combine phenolic compound with methane inhibitors

Results



Effect of phenolic compounds on TGP production when added methane inhibitors during 0 - 24h fermentation

- Phloroglucinol and gallic significantly increased TGP by 9% and 6%, respectively.

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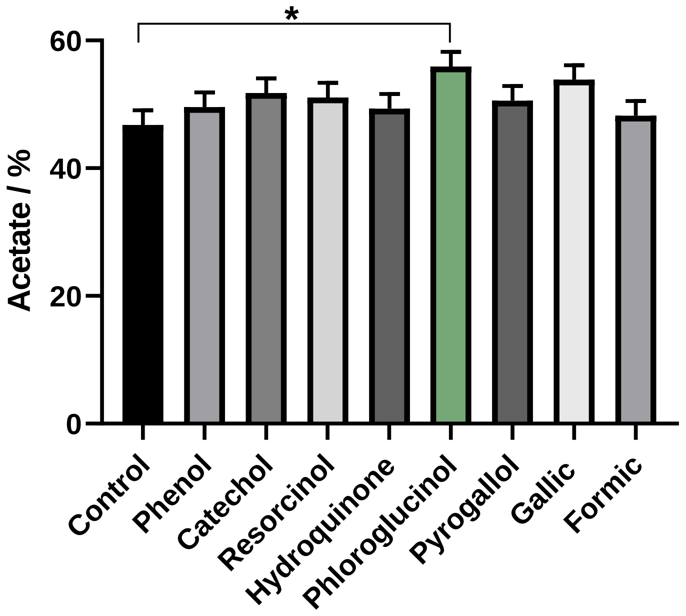
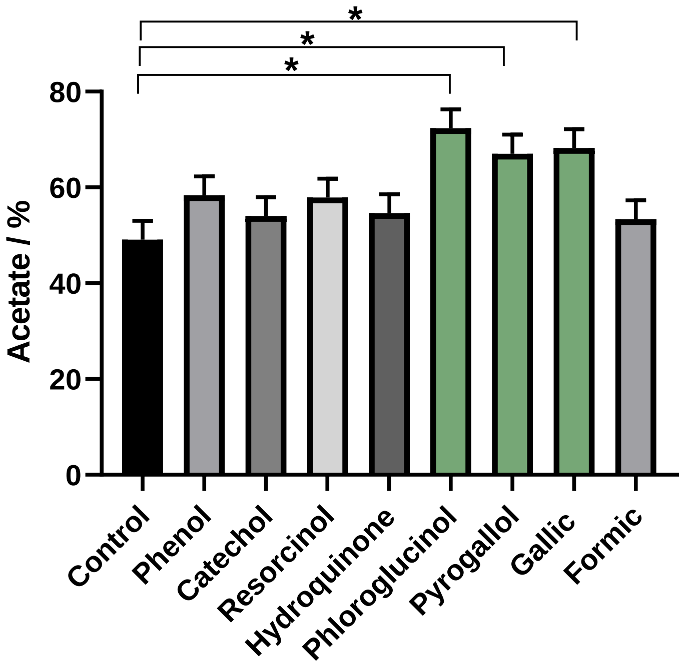
Exp.2 Combine phenolic compound with methane inhibitors

Results



phenolic compounds + 2.5% seaweed

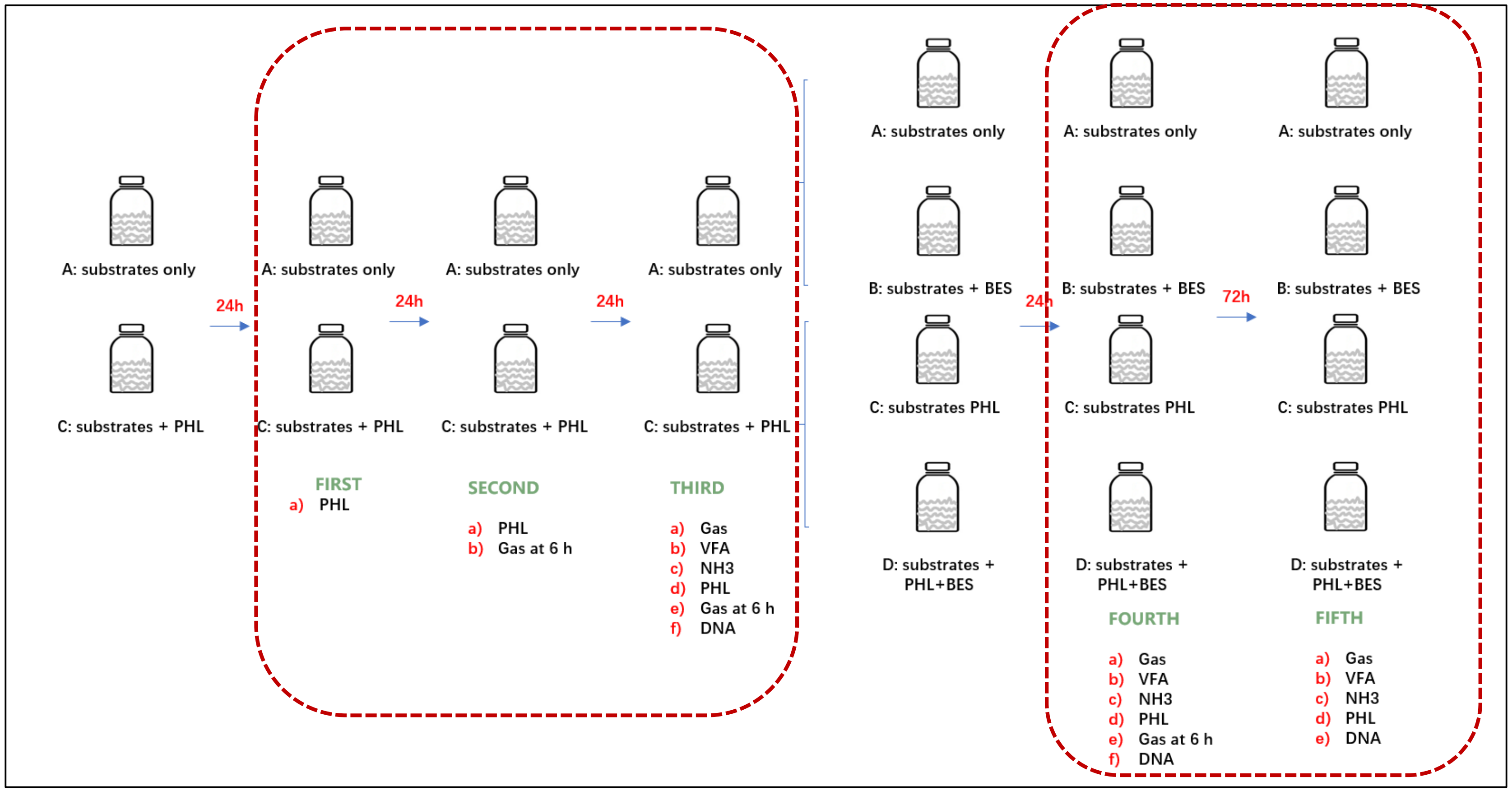


phenolic compounds + BES

- Phloroglucinol, pyrogallol, and gallic acid increased acetate proportion when methane production is inhibited

From Huang 2021

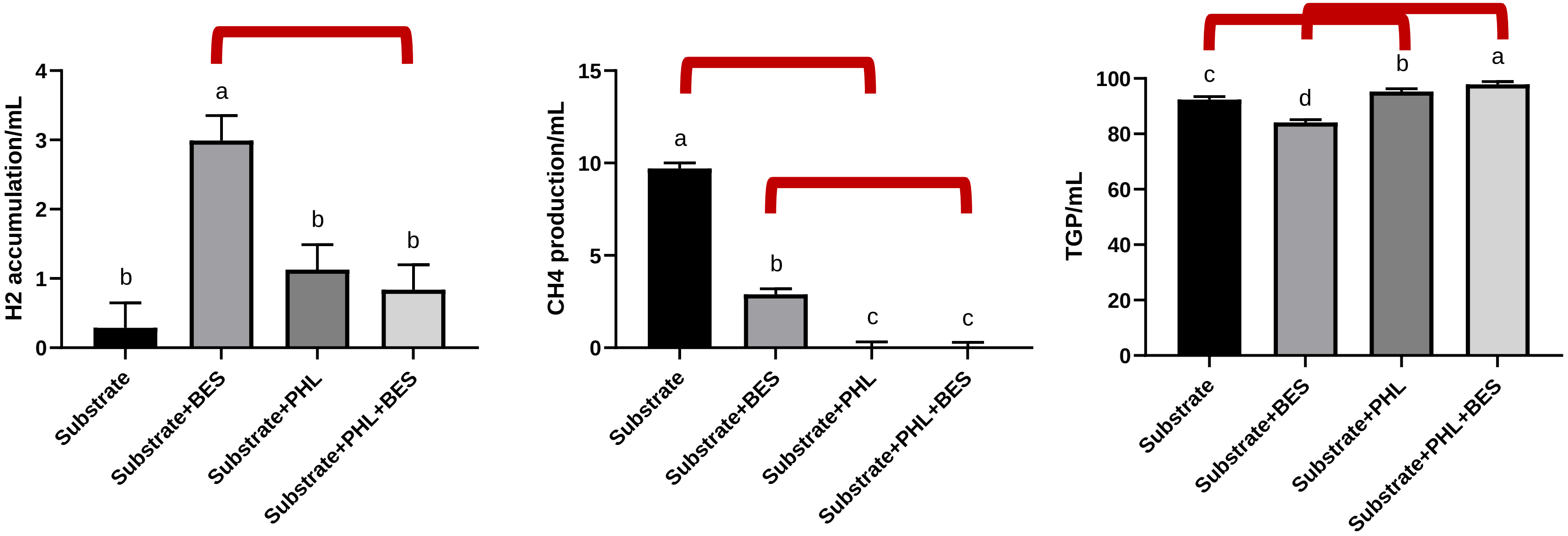
Exp.2.1 Consecutive in vitro incubation



Aimed to study the rumen microbes adapt to phloroglucinol with or without methane inhibitor

From Huang 2021

Exp.2.1-RESULTS



Sequential incubation batch #4

- Substrate+PHL+BES V.S. Substrate+BES: H2 decreased 64%
- Substrate+PHL V.S. Substrate: CH4 decreased 99%
- Substrate+PHL+BES V.S. Substrate+BES: CH4 decreased 100%
- Add PHL increased TGP

From Huang 2021

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Thank you for your attention!

H2020 MASTER
China Scholarship Council
French National Research Agency
project CREDIT (ANR-09-EBIO-009)
H2020 HoloRuminant

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HoloRuminant
Understanding microbiomes of the ruminant holobiont

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