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FERMENTATION, CELL CULTURE AND BIOENGINEERING

Influence of glucose fermentation on CO_2 assimilation to acetate in homoacetogen *Blautia coccoides* GA-1

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Abstract Fermentation of glucose influences CO_2 assimilation to acetate in homoacetogens. *Blautia coccoides* was investigated for a better understanding of the metabolic characteristics of homoacetogens in mixotrophic cultures. Batch cultures of the strain with H_2/CO_2 as a sole carbon source reached an acetate yield of 5.32 g/g dry cell weight after 240 h of incubation. Autotrophic metabolism was inhibited as glucose was added into the culture: the higher the glucose concentration the lower the autotrophic ability of the bacterium. Autotrophy was inhibited by high glucose concentration, probably due to the competition for coenzyme A between the Embden-Meyerhof-Parnas pathway and the Wood-Ljungdahl carbon fixation pathway, the energy (adenosine triphosphate) allocation for synthesis of cell carbon and reduction of CO_2, in combination with the low pH caused by the accumulation of acetate.

Keywords Homoacetogen · Mixotrophic culture · Autotrophic metabolism · Heterotrophic metabolism · Metabolic repression

Introduction

Homoacetogens are autotrophic anaerobes using acetyl-CoA Wood-Ljungdahl carbon fixation pathway (WLP) for energy conservation, acetate production, and biomass formation from CO_2 [7]. Thus, facultative autotrophic growth on H_2/CO_2 or carbon monoxide (CO) is possible [1, 3]. These anaerobes can also utilize organic compounds heterotrophically to produce acetate and form biomass [17, 18, 26]. Acetyl-CoA is the intermediate generated from heterotrophic Embden-Meyerhof-Parnas (EMP) pathway and autotrophic WLP to produce acetate in homoacetogens [19]. These homoacetogens are important to the evolution of life and global carbon cycle [4, 9, 25] and distributed widely in natural environment and very versatile in trophic behavior [6, 25].

In the natural environment, such as soils, aquatic sediments, oil field, wastewater sediments, sewage sludge, etc., heterotrophic activity of homoacetogens is maintained due to the existence of organic substrate, but the autotrophic activity is often overlooked [7]. Though metabolism both in autotrophy and heterotrophy of homoacetogens has been investigated, interaction of the two metabolisms has not been well understood. Furthermore, the interrelationship of the two pathways is species dependent. Braun and Gottschalk reported that *C. aceticum* could grow mixtrophically and the hydrogenase activity in fructose-growth cells amounted to 12 % of that of H_2/CO_2 growth [2]. Therefore, a thorough understanding of how the autotrophic metabolic pathway occurs with the existence of organic substrate is an issue of great importance that needs to be known.

Homoacetogens also inhabit anaerobic digestion systems and have the ability to adjust hydrogen partial pressure (PH_2) and supply acetate to methanogens [12, 27]. Over the period, two-phase anaerobic biological treatment process has been widely used in treating various organic wastewaters of high strength discharged from industries [8, 13]. Biogas released from the acidogenic phase contains a large of H_2 and CO_2 that would contribute to greenhouse

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effect. These greenhouse gases could be introduced to homoacetogens to produce industrial acetate while emission of CO_2 is reduced [14, 19]. A better understanding of the interrelationship between autotrophy and heterotrophy in homoacetogens is vital for acetate synthesis from biogas for effective control of the anaerobic digestion processes.

In previous research, a homoacetogen strain *Blautia coccoides* GA-1 was obtained along with strain CA3 from a continuously stirred tank reactor (CSTR) treating organic wastewater [15]. Both heterotrophic and autotrophic metabolism coexisted in *B. coccoides* when mixotrophically cultured [5, 10, 11, 24]. However, the nutritional requirements and growth of this homoacetogen were not ascertained. In this paper, strain GA-1 was cultured in heterotrophic, autotrophic, and mixotrophic conditions, and the strain's growth and glucose influence on autotrophic synthesis of the acetate were investigated.

Materials and methods

Microorganism

The homoacetogen, *B. coccoides* GA-1, was isolated from a CSTR fed with molasses wastewater [15]. It had been identified as a strictly anaerobic, gram-positive, non-sporeforming bacterium and was capable to grow in temperatures ranging from 20 to 50 °C. Only acetate could be detected in the broth with glucose or H_2/CO_2 as sole carbon source, and the producing rate reached 8.92 mg/(L·h) with H_2/CO_2 as sole carbon source [15].

Media

The basal medium contained the following per 1 L distilled water: K_2HPO_4, 0.348 g; KH_2PO_4, 0.227 g; NH_4Cl, 0.5 g; $MgSO_4{\cdot}7H_2O$, 0.5 g; $CaCl_2{\cdot}2H_2O$, 0.25 g; NaCl, 2.25 g; Yeast extract, 0.5 g; $NaHCO_3$, 1.0 g; cysteine, 0.5 g; Vitamin solution, 10 mL; Trace element solution, 1 mL. The trace element solution contained per 1 L distilled water the following: $ZnSO_4{\cdot}7H_2O$, 0.18 g; $CaCl_2{\cdot}2H_2O$, 0.1 g; $CuSO_4{\cdot}5H_2O$, 0.01 g; $MnSO_4{\cdot}H_2O$, 0.5 g; $CoSO_4{\cdot}7H_2O$, 0.18 g; $KAl(SO_4)_2{\cdot}12H_2O$, 0.02 g; $NiCl_2{\cdot}6H_2O$, 0.03 g; $Na_2SeO_3{\cdot}5H_2O$, 0.3 mg; $Na_2MoO_4{\cdot}2H_2O$, 0.01 g; H_3BO_3, 0.01 g. Vitamin solution contained per 1 L distilled water the following: Biotin, 2.0 mg; Folic acid, 2.0 mg; Pyridoxine–HCl, 10.0 mg; Thiamine-HCl·$2H_2O$, 5.0 mg; Riboflavin, 5.0 mg; Nicotinic acid, 5.0 mg; D-Ca-pantothenate, 5.0 mg; Vitamin B_{12}, 0.1 mg; p-Aminobenzoic acid, 5.0 mg; Lipoic acid, 5.0 mg. The pH was adjusted to 7.0 with NaOH solution of 2 mol/L. The medium was boiled under a stream of O_2-free N_2 gas and cooled to room temperature. Each anaerobic bottle was dispensed with 50 mL basal medium and sterilized by autoclaving at 121 °C for 20 min. Prior to culture inoculation, 0.2 mL of 5 % (*w/v*) $NaHCO_3$ and 0.05 mL of 25 % (*w/v*) $Na_2S{\cdot}9H_2O$ were injected into the 50 mL presterilized medium. A concentrated anaerobic sterile stock solution of glucose as a substrate for heterotrophic and mixotrophic cultures was injected to a desired concentration.

Inoculum and culturing condition

Butyl rubber-stoppered anaerobe bottles were used as anaerobic reactors, each of which contained 55 mL of liquid and 85 mL of headspace inflated with gas. Strain GA-1 was enriched in the basal medium as 200 mg/L glucose was added, with H_2/CO_2 (*v/v*: 4:1) as gas phase. The growth was carried out at 37 °C with an initial pH of about 7.0 in a gyratory incubator-shaker at 130 rpm for 72 h. Bacterial suspension with a dry cell weight concentration of 0.142–0.186 g/L was prepared and used as inoculum. Each anaerobic bottle containing 50 mL medium was inoculated with 5 mL of the bacterial suspension. H_2/CO_2 was used as gas phase and substrates simultaneously in autotrophic culturing, while glucose was the carbon source with N_2 as gas phase in the case of heterotrophic culturing. In mixtrophic cultures, carbon source in the media was glucose and the gas phase was H_2/CO_2. Acetate and biomass produced from the basal medium were measured as control and was subtracted from the corresponding tests. All of the tests were carried out in triplicate at 37 °C in a gyratory incubator-shaker (130 rpm).

Analytical methods

Pressure in the anaerobic bottles was balanced to normal pressure by injecting N_2 with syringes. The amount of injected N_2 was quantified since H_2/CO_2 was utilized by the homoacetogens. At termination, 0.5 mL gas in headspace of each bottle was sampled separately and the fraction of H_2 and CO_2 was analyzed by a gas chromatograph (SP-6800A, Shandong Lunan Instrument Factory, China) equipped with a thermal conductivity detector (TCD) and a 2-m stainless column packed with Porapak Q (60/80 mesh) [16]. Temperatures of the injector, the column and the TCD were 80, 50, and 80 °C, respectively.

Liquid samples, 1.5 mL for each culture, were taken separately with sterile syringes for determination of pH (DELTA 320, Mettler Toledo, US) and acetate. Acetate was measured by another gas chromatography (SP6890, Shandong Lunan Instrument Factory, China) equipped with an RTX-Stabilwax glass column (30 m × 0.32 mm × 1 μm) and an aflame ionization detector (FID). The operational temperatures of the injection port, the oven, and the detector were 210, 165, and 210 °C, respectively. Nitrogen was

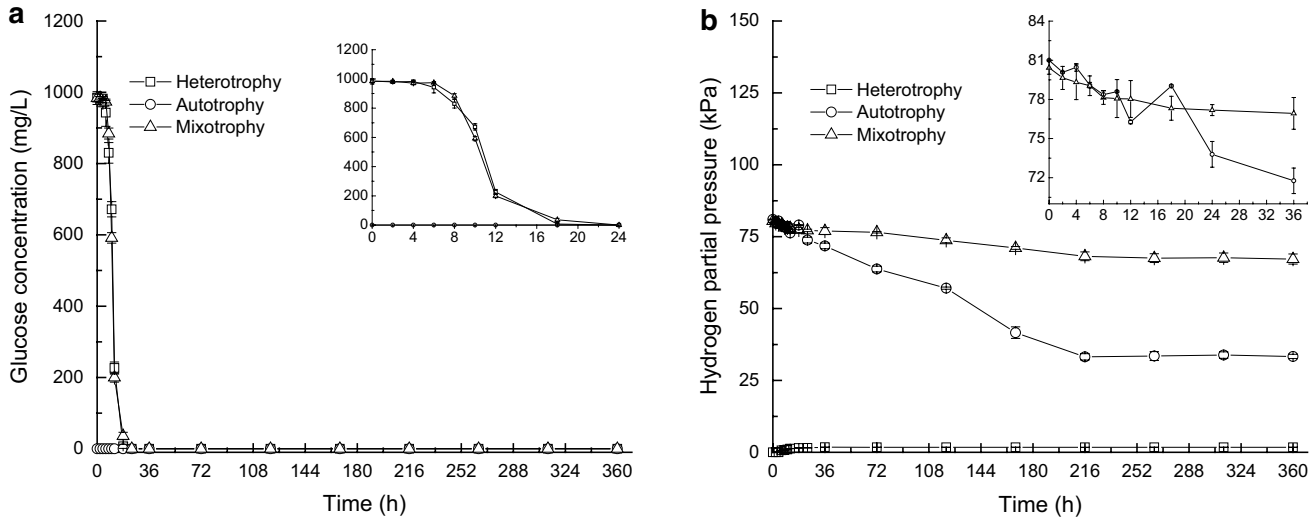

Fig. 1 Glucose concentration (**a**) and hydrogen partial pressure (**b**) in autotrophic, heterotrophic, and mixotrophic cultures of the *B. coccoides* GA-1

used as carrier gas, with a 0.75-MPa column head pressure. The split ratio was 1:50. For acetate determination, 1 mL of the liquid sample was centrifuged at 13,000 rpm for 4 min. pH of the supernatant was adjusted to about 3 by adding 25 % H_3PO_3, and then 1 μL was sampled and injected into the gas chromatography. Glucose in broth and liquid suspension was measured following the standard method [23].

Optical density (OD) of bacterial suspension was measured by a photoelectric spectrophotometer (UV-2450, Shimadzu, Japan) at 600 nm (OD_{600}) in accordance with standard methods [23]. Standard curve of OD_{600} to biomass of dry cell weight was established and the biomass was evaluated by the equation of linear regression.

Results

Confirmation of nutritional type of strain GA-1

In order to confirm the characteristics of *B. coccoides* GA-1 in trophic modes, metabolic activity of the strain was evaluated in autotrophic, heterotrophic, and mixotrophic cultures with consumption of glucose and H_2 as indicators. As shown as Fig. 1a, the glucose both in heterotrophic and mixotrophic cultures was completely degraded within 24 h. The PH_2 in mixotrophic cultures decreased very slowly before the glucose fermentation came to the end and was accelerated after the glucose fermentation process (Fig. 1b). The decrease in PH_2 indicated the occurrence of autotrophic metabolism. An ultimate yield of acetate in autotrophic, heterotrophic, and mixotrophic cultures was detected to be 331.6, 352.2, and 498.6 mg/L, respectively. The results indicated that strain GA-1 was mutable in nutritional types. It could grow autotrophically, heterotrophically, and mixotrophically to produce acetate and accumulate biomass.

It was very interesting to find that there was H_2 accumulation in the heterotrophic cultures with a corresponding PH_2 of about 1.7 kPa (Fig. 1b). This result suggested an imbalance in fluxes of reducing equivalents between the EMP pathway and the WLP, because the endogenous synthesis of H_2 from the excess reducing equivalents synthesized by the EMP pathway could not be entirely utilized by the WLP [8, 25, 26]. It was critical to find out the reason for this imbalance for understanding the influence of heterotrophy on autotrophy in mixotrophic cultures of homoacetogen *B. coccoides* GA-1.

Influence of glucose concentration on autotrophy of strain GA-1 in mixtrophic cultures

The growth of Strain GA-1 and acetate production from H_2/CO_2 was investigated in mixotrophic cultures using autotrophic and heterotrophic cultures as controls. An accumulation of H_2 was observed in the heterotrophic cultures and this accumulation increased whiles glucose concentration exceeded 600 mg/L (Fig. 2a). With no glucose in the broth, the PH_2 in the autotrophic cultures decreased from the initial 73.06 to 33.89 kPa (Fig. 2b), and an acetate yield of about 349.16 mg/L was obtained (Fig. 3a), with a biomass yield of about 65.24 mg/L (Fig. 3b), indicating a better autotrophic growth of the strain.

All of the PH_2 in mixotrophic cultures observably decreased after fermenting for 240 h, and the more the initial glucose the higher the PH_2 (Fig. 2b). The acetate yields in mixotrophic cultures increased following the enhancement

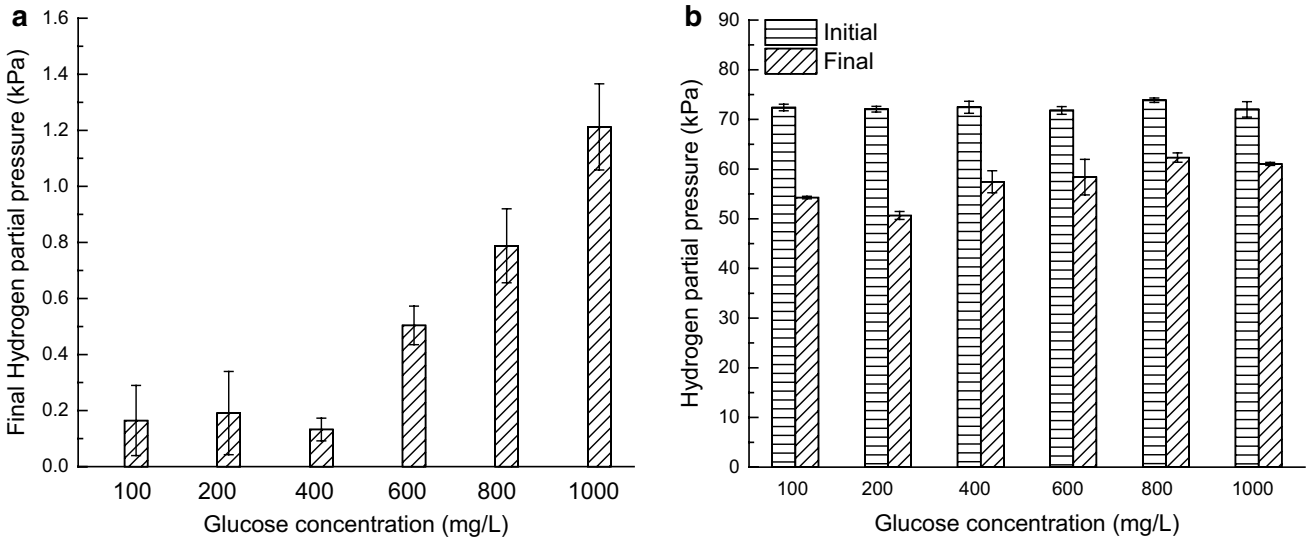

Fig. 2 Changes of hydrogen partial pressure in heterotrophic cultures (**a**) and mixotrophic cultures (**b**) of the *B. coccoides* GA-1 following the increase of initial glucose concentration

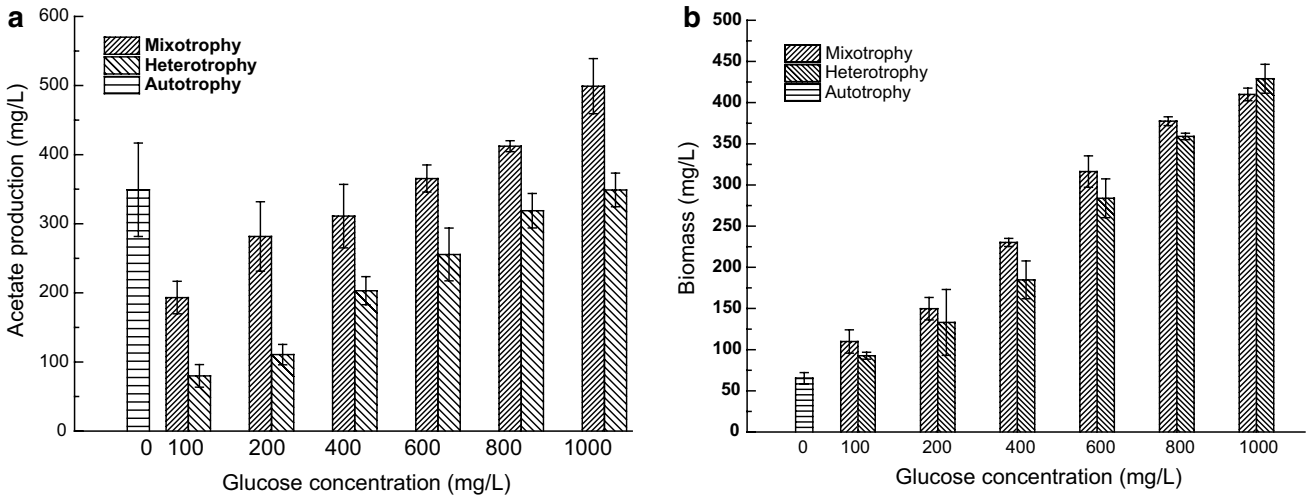

Fig. 3 Acetate production (**a**) and biomass formation (**b**) in autotrophic (with no glucose in the cultures), heterotrophic and mixotrophic cultures of the *B. coccoides* GA-1 following the increase of initial glucose concentration

of initial glucose concentration (Fig. 3a). Though acetate yield in the mixotrophic cultures was improved from 193.19 to 311.05 mg/L when initial glucose concentration increased from 100 to 400 mg/L, it was comparatively lower than that produced in autotrophic cultures. This was possibly resulted from the assimilation of glucose into biomass (Fig. 3b).

As illustrated in Fig. 3b, the biomass was about 65.24 mg/L in autotrophic cultures, but remarkably increased to about 109.91 mg/L when 100 mg/L glucose was added into the culture. When the concentration of initial glucose increased from 200 to 1000 mg/L in mixotrophic cultures, the biomass increased from 149.76 to 409.94 mg/L. The lower acetate yield and higher biomass yield compared to that in autotrophic control cultures (Fig. 3) indicated that most glucose had been assimilated into biomass.

Discussion

Repression of glucose fermentation on the autotrophic growth in mixotrophic cultures

Batch cultures (Figs. 1, 2 and 3) showed that strain GA-1 could grow autotrophically, heterotrophically, and

mixotrophically. The performance in biomass, hydrogen consumption, and acetate yield in mixotrophic and heterotrophic cultures are presented in Table 1. The results revealed that biomass in mixotrophic cultures and heterotrophic cultures were significantly enhanced by the increasing glucose concentration. The specific acetate yield of biomass, ranging from 0.79 to 0.94 mg/mg dry-cell, in heterotrophic cultures seemed to be marginally affected by the glucose concentration. On the contrary, the specific acetate yield in mixotrophic cultures decreased gradually from 1.79 to 1.02 mg/mg dry-cell when the initial glucose concentration increased from 100 to 1000 mg/L. The decrease in specific acetate yield with the increase in glucose concentration indicated that glucose had repressed the autotrophic growth that consumed H_2/CO_2; thus the higher the glucose concentration the stronger the repression. This is also indicated by the decrease in specific hydrogen consumption of the biomass. The specific hydrogen consumption of biomass was about 0.41 mmol/mg in autotrophic cultures. In mixotrophic cultures, the specific hydrogen consumption of biomass decreased from 0.12 to 0.02 mmol/mg when glucose concentration increased from 100 to 1000 mg/L.

As illustrated in Table 1, the specific biomass yield by glucose consumed in mixtrophic cultures decreased from 2.75 to 1.02 mg/mg when initial glucose concentration increased from 100 to 1000 mg/L, though the biomass increased from 109.91 to 409.94 mg/L (Fig. 3b). This result suggested that glucose was utilized less efficiently for biomass synthesis in mixotrophic cultures, possibly due to the added maintenance burden arising out of the inhibitory effects of acetate accumulation and increased cell density.

Figure 4 shows the autotrophic and heterotrophic metabolism pathway of CO_2 and glucose in homoacetogens [6, 18, 25]. In heterotrophic metabolism [13], homoacetogens utilize hexoses to generate pyruvate by EMP pathway. Pyruvate is then converted to acetyl-CoA by substrate-level phosphorylation [6]. In autotrophic metabolism [6, 13], CO_2 is reduced into acetyl-CoA through WLP. The reducing equivalents ([H]) for CO_2 reduction is provided by the EMP pathway of hexose [21]. The acetyl-CoA in EMP pathway or/and WLP is finally converted to cell carbon and acetate.

Reduction of CO_2 in heterotrophic metabolism is also achieved by WLP. The reducing equivalents should come from activation of the exogenetic H_2. Ferredoxin, hydrogenase and [FeFe]-hydrogenase complex have been identified in acetogens [13, 21]. Exogenetic H_2 is diffused into cells by concentration gradient and oxidized to [H] catalyzed by ferredoxin together with hydrogenase [13]. With the [H] from the exogenetic H_2, CO_2 is reduced into acetyl-CoA through WLP.

As showed in Fig. 1, the performance of strain GA-1 in mixotrophic cultures could be divided into two phases, with glucose fermentation process coming up first and followed by autotrophic metabolism. Though the first phase was dominated by glucose fermentation, autotrophic metabolism occurred that resulted in a slight decrease in PH_2. Comparatively, the marginal decrease in PH_2 during the first phase suggested that the autotrophic metabolism had been repressed remarkably. The following three mechanisms were suggested to be responsible for the repression: (1) the competition for Coenzyme A (CoA) between EMP and WLP, (2) the energy (ATP) allocation for synthesis of cell carbon and reduction of CO_2, and (3) the inhibition of low pH caused by the accumulation of acetate.

In mixtrophic cultures, the homoacetogen could grow rapidly by glucose fermentation (Fig. 3; Table 1). Reduction of CO_2 could occur in mixotrophic cultures even with a glucose concentration as high as 1000 mg/L, but this was remarkably repressed within the glucose fermentation process (Fig. 1). CoA was a key enzyme not only for the decarboxylation of pyruvate in EMP pathway, but also for the synthesis of acetyl-CoA from methyl- corrinoidiron sulfur protein (CFeSP) and CO in WLP (Fig. 4) [21, 25]. In other words, CoA was shared by EMP pathway and WLP for formation of acetyl-CoA. Within the glucose fermentation process, most of the CoA was used by EMP glycolysis while less CoA could be acquired by WLP, resulting in a repression on the autotrophic metabolism of strain GA-1 [5, 6, 18].

Both the autotrophic and heterotrophic metabolism of homoacetogens can yield energy during reductive synthesis of acetate and biomass [5, 21]. A net yield of 2 mol adenosine triphosphate (ATP) was given when 1 mol glucose is converted to 2 mol acetate by EMP pathway, while no net ATP is yielded in WLP (1 mol ATP consumed when CO_2 is conserved to formate, while 1 mol ATP produced when synthesis of acetate from acetyl-CoA) [5, 7, 18]. Metabolic flux of WLP should be enhanced by the ATP from the EMP pathway [5, 13]. The key issue is whether this energy could be transported into the WLP and utilized as well. Batch cultures of strain GA-1 in mixotrophic cultures showed that biomass concentration had been remarkably enhanced by the addition of glucose (Table 1), and CO_2 reduction within glucose fermentation process was repressed evidently (Fig. 1). It became apparent that less ATP could be obtained by the WLP because most of the ATP produced by EMP synthesis had been used for synthesis of cell carbon. Therefore, synthesis of acetate from the exogenetic H_2/CO_2 was restricted within glucose fermentation process.

As showed in Fig. 1, the autotrophic metabolism in mixotrophic cultures mainly occurred after the glucose fermentation process, and the acetate accumulation became more

Table 1 Fermentation production and hydrogen consumption in heterotrophic and/or mixotrophic cultures of *B. coccoides* GA-1

Glucose concentration (mg/L)	Heterotrophic cultures				Mixotrophic cultures					
	Biomass production (mg)	Acetate yield (mg)	Acetate yield by biomass (mg/mg)	Biomass yield on glucose (mg/mg)	Biomass yield (mg)	Hydrogen consumption (mmol)	Acetate yield (mg)	Hydrogen consumption by biomass (mmol/mg)	Acetate yield by biomass (mg/mg)	Biomass yield on glucose (mg/mg)
0[a]					3.59 ± 0.38	1.69 ± 0.04	19.20 ± 3.72	0.41 ± 0.06	5.32 ± 0.50	–
100	5.10 ± 0.23	4.39 ± 0.90	0.87 ± 0.21	2.32 ± 0.10	6.04 ± 0.78	0.71 ± 0.04	10.63 ± 1.29	0.12 ± 0.01	1.79 ± 0.44	2.75 ± 0.35
200	7.32 ± 2.20	6.09 ± 0.81	0.87 ± 0.21	1.66 ± 0.50	8.24 ± 0.75	0.83 ± 0.00	17.00 ± 1.25	0.11 ± 0.00	2.18 ± 0.22	1.78 ± 0.05
400	10.16 ± 1.26	9.34 ± 3.92	0.94 ± 0.46	1.15 ± 0.14	12.66 ± 0.27	0.59 ± 0.06	17.11 ± 2.52	0.05 ± 0.00	1.35 ± 0.22	1.44 ± 0.03
600	15.61 ± 1.30	12.22 ± 2.74	0.79 ± 0.24	1.18 ± 0.10	17.40 ± 1.05	0.54 ± 0.11	20.10 ± 1.08	0.03 ± 0.01	1.16 ± 0.02	1.32 ± 0.08
800	19.74 ± 0.21	17.54 ± 1.37	0.89 ± 0.07	1.12 ± 0.01	20.76 ± 0.29	0.48 ± 0.05	22.67 ± 0.43	0.02 ± 0.00	1.09 ± 0.03	1.18 ± 0.02
1000	23.59 ± 0.97	19.19 ± 1.34	0.82 ± 0.08	1.07 ± 0.04	22.55 ± 0.42	0.48 ± 0.03	27.45 ± 2.19	0.02 ± 0.00	1.02 ± 0.11	1.02 ± 0.02

[a] With no glucose in the mixotrophic cultures, i.e. autotrophic cultures

observable following the increasing glucose concentration (Fig. 3a). The low pH resulting from the acetate accumulation by glucose fermentation was certain to repress the autotrophic activity of strain GA-1 in mixotrophic cultures. It was found that the pH in mixotrophic cultures drop down to below 5.5 (not otherwise provided) due to acetate accumulation when glucose concentration was over 400 mg/L. It has been reported that no homoacetogenesis could be observed at thermophilic conditions with initial pH 5.5 [17, 25].

Accumulation of hydrogen in the heterotrophic cultures

It was found that there was H_2 accumulation in the heterotrophic cultures (Fig. 2a), especially when the initial glucose concentration was above 600 mg/L (Table 1). Obviously, the endogenous reducing equivalents synthesized by the EMP pathway could not be entirely utilized by the WLP, indicating an imbalance in fluxes of reducing equivalents between the two pathways. This imbalance had been also indicated in other homoacetogens, such as *Clostridium thermoaceticum* [22, 28], *Ruminococcus albus* [29], *Eubacterium limosum* [20], and *Acetobacterium woodii* [2].

It is known that 2 mol of acetyl-CoA, 2 mol of ATP, 2 mol of CO_2, and 8 electrons are generated from each mole of glucose during glycolysis (reaction 1). Without a mechanism for CO_2 reassimilation, CO_2 is lost, and much of the reducing equivalent pool is oxidized by hydrogenase activity to release H_2 [8]. In heterotrophic cultures, ATP, CO_2, and [H] are needed to start up the autotrophic WLP. During glycolysis, 2 mol pyruvate, 2 mol ATP, and 4 electrons are generated from 1 mol glucose (reaction 2), but no CO_2 produced. CO_2 is produced only when decarboxylation of pyruvate occurs (reaction 3):

$$1\ \text{Glucose} \rightarrow 2\ \text{Acetyl-CoA} + 2\ \text{ATP} + 2\ CO_2 + 8[\text{H}] \quad (1)$$

$$1\ \text{Glucose} \rightarrow 2\ \text{Pyruvate} + 2\ \text{ATP} + 4[\text{H}] \quad (2)$$

$$2\ \text{Pyruvate} \rightarrow 2\ \text{Acetyl-CoA} + 2\ CO_2 + 4[\text{H}] \quad (3)$$

It has been reported that the reductive decarboxylation of pyruvate may be a rate-limiting reaction for growth on glucose alone [20]. This means that heterotrophic growth would occur previous to autotrophic growth. Before achieving a flux balance between the EMP pathway and the WLP, the 4 mol [H] produced by the glycolysis are excess reducing equivalents and oxidized by hydrogenase activity resulting in the release of H_2. When the balance is established in the heterotrophic cultures, all of the 8 [H] synthesized by the EMP pathway can be entirely utilized by the WLP [8, 25], and no changes in PH_2 could be found anymore in the heterotrophic cultures of *B. coccoides* GA-1 after the glucose fermentation process (Fig. 1).

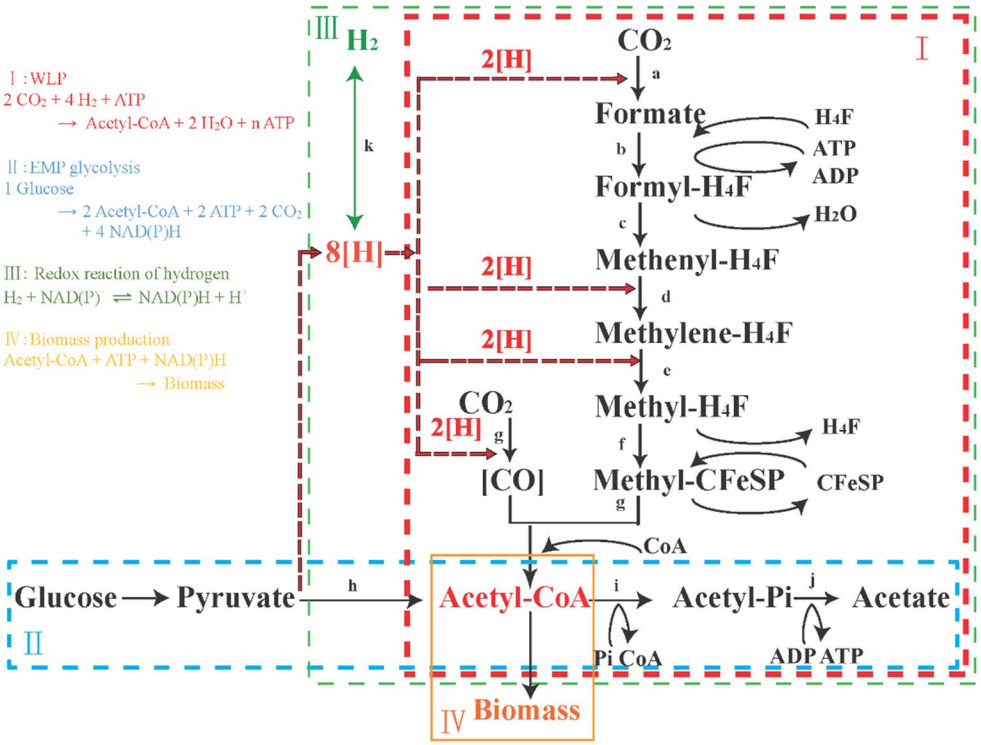

Fig. 4 Metabolic pathway of homoacetogens (Modified from Saady, N.M.C. 2013). Parenthetical characters identify enzymes that catalyze the indicated reactions:*a* formate dehydrogenase; *b* formyltetrahydrofolate synthetase; *c* formyltetrahydrofolate cyclohydolase; *d* methylenetetrahydofolate dehydrogenase; *e* methylenetetrahydofolate reductase; *f* methyltransferase; *g* CO dehydrogenase/acetyl-CoA synthase (CODH/ACS); *h* pyruvate dehydrogenase; *i* phosphotransacetylase; *j* acetate kinase; *k* [FeFe]-hydrogenase

Conclusion

The heterotrophic effect on autotrophic metabolism in homoacetogen *B. coccoides* GA-1 was investigated by batch cultures. An initial heterotrophic consumption of glucose by the coordinated activity of the EMP pathway and WLP to produce acetate and biomass was found in mixotrophic cultures, but the autotrophic metabolism had been repressed remarkably. Competition for CoA between EMP and WL pathways, ATP allocation for synthesis of cell carbon and reduction of CO_2, and inhibition of low pH should be responsible for the repression, while reduced hydrogen carrier levels must have impact on the bacterial growth too. Autotrophic CO_2 fixation after all the glucose was consumed, where the extent of this is inhibited possibly by low pH caused by the accumulation of acetate.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human participants This article does not contain any studies with human participants performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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