读书报告

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One-step process for production of *N*-methylated amino acids from sugars and methylamine using recombinant *Corynebacterium glutamicum* as biocatalyst

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使用重组谷氨酸棒杆菌作为生物催化剂从糖和甲胺生产　甲基化氨基酸的一步法

CONTENT

I M M

R D C

I

氨基酸的N-甲基化

氨基酸的N-甲基化发生在细菌和真核生物中。

I , N^5- L-

(C ,).

N-

. . ,

D .

与非甲基化肽相比，含有N-甲基氨基酸的肽在蛋白水解和增加膜通透性方面具有更高的稳定性。

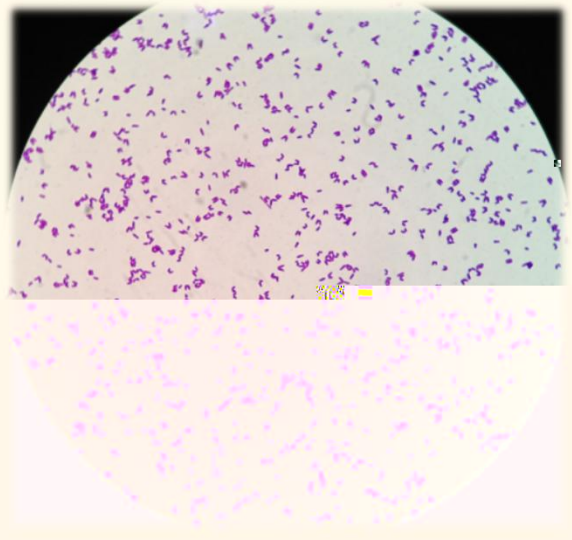
I

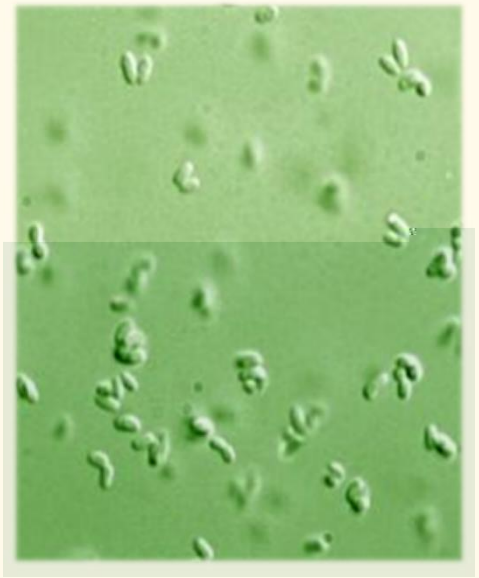
I , - (MMA)
N- .

-
MMA .


A
O
OH
O
NH2
NADPH NADP+
DpkA
NH
OH
O
pyruvate
丙酮酸
methylamine
甲基丙氨酸
NMeAla
N-甲基丙氨酸

谷氨酸棒杆菌





F , *C. glutamicum*

(& F , B R , 2005).

F

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C. glutamicum

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M C.

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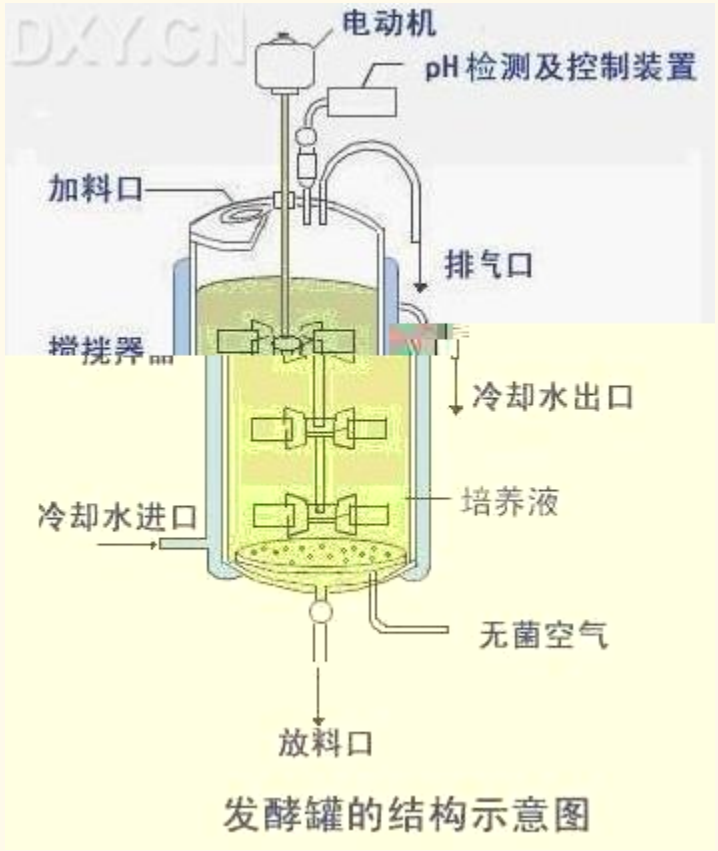
B

Bacterial trains and vectors used in this study.

Strains and vectors	Description	Source
Strains		
WT	*C. glutamicum* wild type, ATCC13032	American Type Culture Collection
ELB-P	WT carrying deletions *ΔaceE Δpqo ΔldhA ΔC-T ilvN ΔalaT ΔavtA*	31
NMeAla1	WT carrying deletions *ΔaceE Δpqo ΔldhA ΔC-T ilvN ΔalaT ΔavtA* and vector pVWEx1-*dpkA*	This work
Plasmids		
pVWEx1	Kan^R, *C. glutamicum/E. coli* shuttle vector (P_{tac}, *lacI*, pHM1519 $oriV_{C.g.}$)	85
pEKEx3	$Spec^R$, *C. glutamicum/E. coli* shuttle vector (P_{tac}, *lacI*, pBL1 $OriV_{C.g.}$)	86
pECXT99A	Tet^R, *C. glutamicum/E. coli* shuttle vector (P_{trc}, *lacI*, pGA1 $OriV_{C.g.}$)	87
pVWEx1-*dpkA*	Kan^R, pVWEx1 overexpressing *dpkA* from *P. putida* KT2440 and change of start codon GTG to ATG	This work
pEKEx3-$xylA_{Xc}$-$XylB_{Cg}$	$Spec^R$, pEKEX3 overexpressing *xylA* from *Xanthomonas campestris* SCC1758 and *xylB* from *C. glutamicum* ATCC 13032	45
pECXT99A-*araBAD*	Tet^R, pECXT99A overexpressing *araBAD* from *E. coli* MG1655	This work
pECXT99A-*amyA*	Tet^R, pECXT99A overexpressing *amyA* from *Streptomyces griseus* IMRU3570	42

F -B



发酵罐的结构示意图

第一次进料阶段

26.7 L ^{1}乙酸钾溶液（总体积：500 L）
取决于相对溶解氧饱和度，当 DO 信号升至60%以上时激活，当 DO 感觉低于60%时停止。

第二次进料阶段（22 后）

初次加入164 L $^{-1}$葡萄糖和84 L $^{-1}$ MMA（总体积：1000 L）然后以12.3 L $^{-1}$进行线性进料。
在最初的24小时内每隔2小时自动取样，然后每8小时取样，并冷却至4 直至分析。

R D

Corynebacterium glutamicum NM A

:

50 M MMA

50 M NM A

50 M MMA

50 M NM A

30 M 17 M

C.

MMA NM A

( ).

C NM A

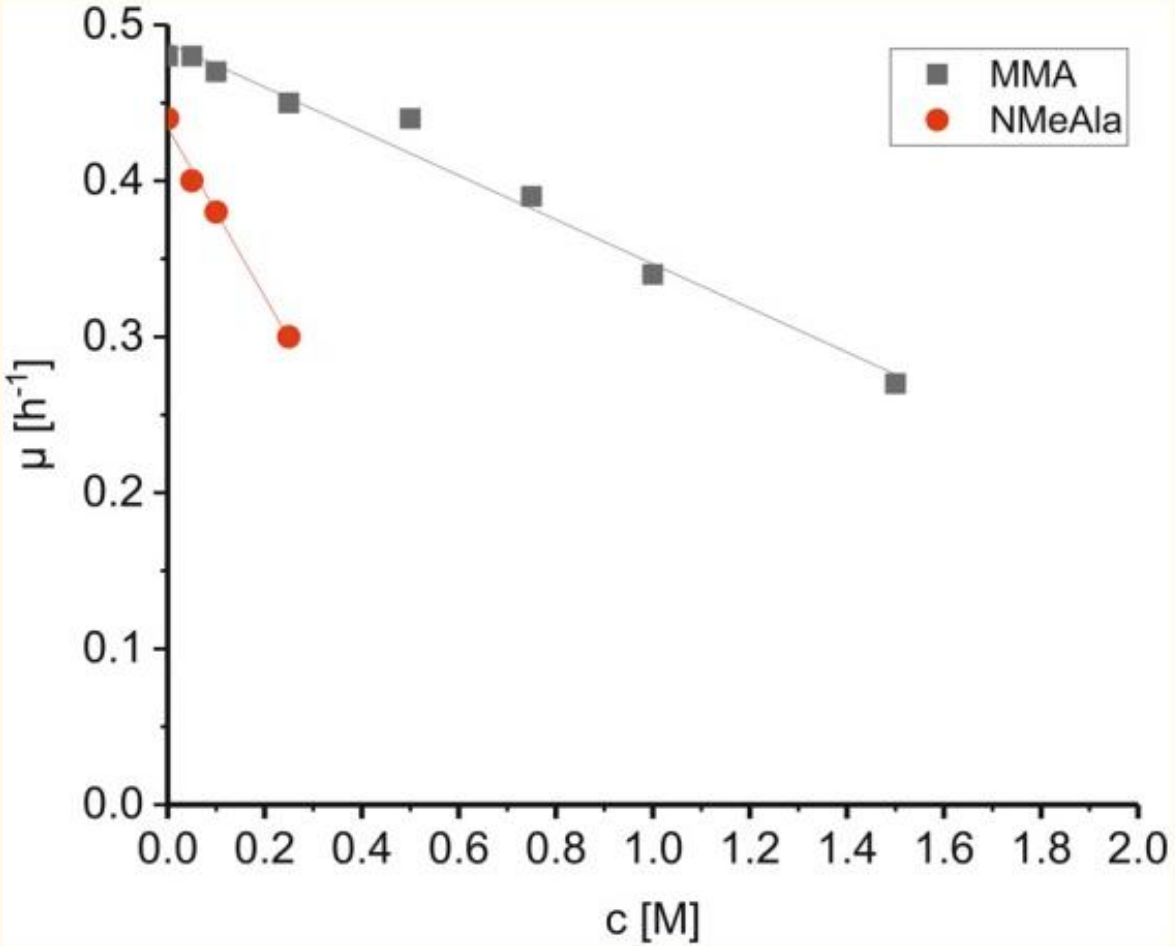


Figure 2. Growth rates of *C. glutamicum* wild type in the presence of varying concentrations of MMA or NMeAla. *C. glutamicum* wild type was grown in presence of increasing MMA (0.05 M to 1.5 M) or NMeAla (0.05 M to 0.25 M) concentrations and specific growth rates were determined. Half maximal growth rates were obtained by extrapolation.

-

1.8 M MMA

0.4 M NM A , .

C NM A

Supplementary Table: Differential gene expression of *C. glutamicum* wild type grown in glucose containing CGXII minimal medium supplemented with 250 mM MMA or 125 mM $(NH_4)_2SO_4$.

Gene ID[ab]	Gene name[b]	Gene annotation[b]	mRNA level (MMA/$(NH_4)_2SO_4$)[c]
cg0759	*prpD2*	2-Methylcitrate dehydratase, involved in propionate catabolism	5.0
cg0762	*prpC2*	2-Methylcitrate synthase, involved in propionate catabolism	2.7
cg0801	-	Hypothetical protein	2.7
cg2566	-	Putative secreted protein	0.3
cg3402	-	Putative Hg_2^+ permease, MerTP-family	2.7

[a] Genes shown are sorted to their identifiers.

[b] Gene ID, name and annotation are according to BX927147

[c] Differential gene expression as calculated for two biological replicates. Values listed were selected for $P < 0.05$ and at least twofold change of the RNA level.

M C. NM A .

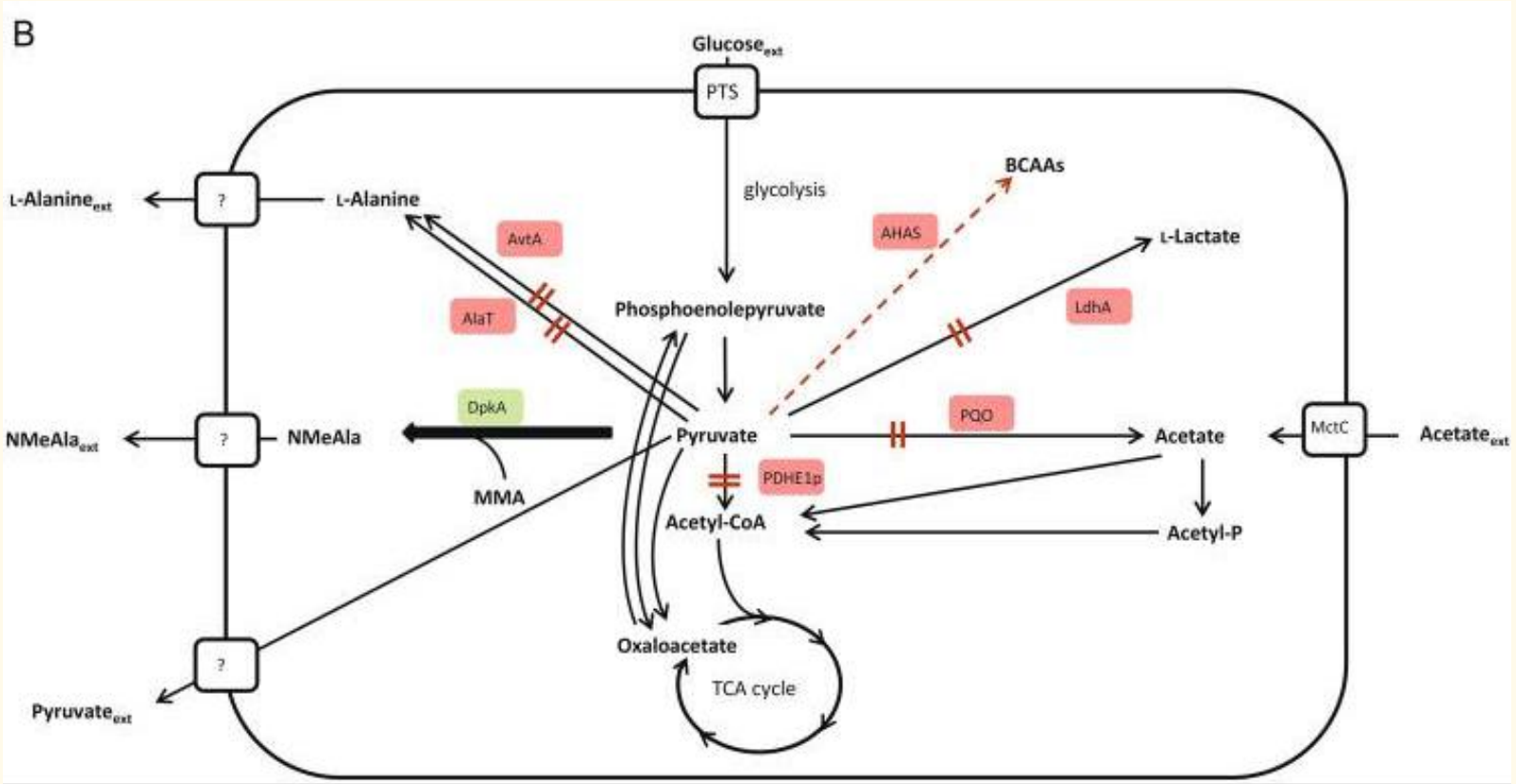


Figure 1. Schematic overview of the reaction catalyzed by DpkA (**A**) and its integration into the central carbon metabolism in *C. glutamicum* NMeAla1 (**B**). The gene deletions for improved pyruvate production are shown by black arrows with red double bars: deletion of *aceE* (encoding PDHE1p, the E1p subunit of the PDHC) and *pqo* (encoding pyruvate-quinone oxidoreductase, PQO) and both genes coding both major enzymes for L-alanine supply by pyruvate aminotransferases (*alaT* and *avtA*, encoding the alanine aminotransferase AlaT and the valine-pyruvate aminotransferase AvtA, respectively) were deleted. In addition, the acetohydroxyacid synthase (AHAS) activity was downregulated by deletion of the C-terminal part of *ilvN* (small subunit of AHAS) shown by [illegible]. Enzymes highlighted by red background indicate missing or down regulated enzymes The thick arrow displays the NMeAla formation by heterologous expressed *dpkA* from *P. putida* KT2440 coding for the *N*-methylated amino acid dehydrogenase DpkA (green shadowed Enzyme).

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I -

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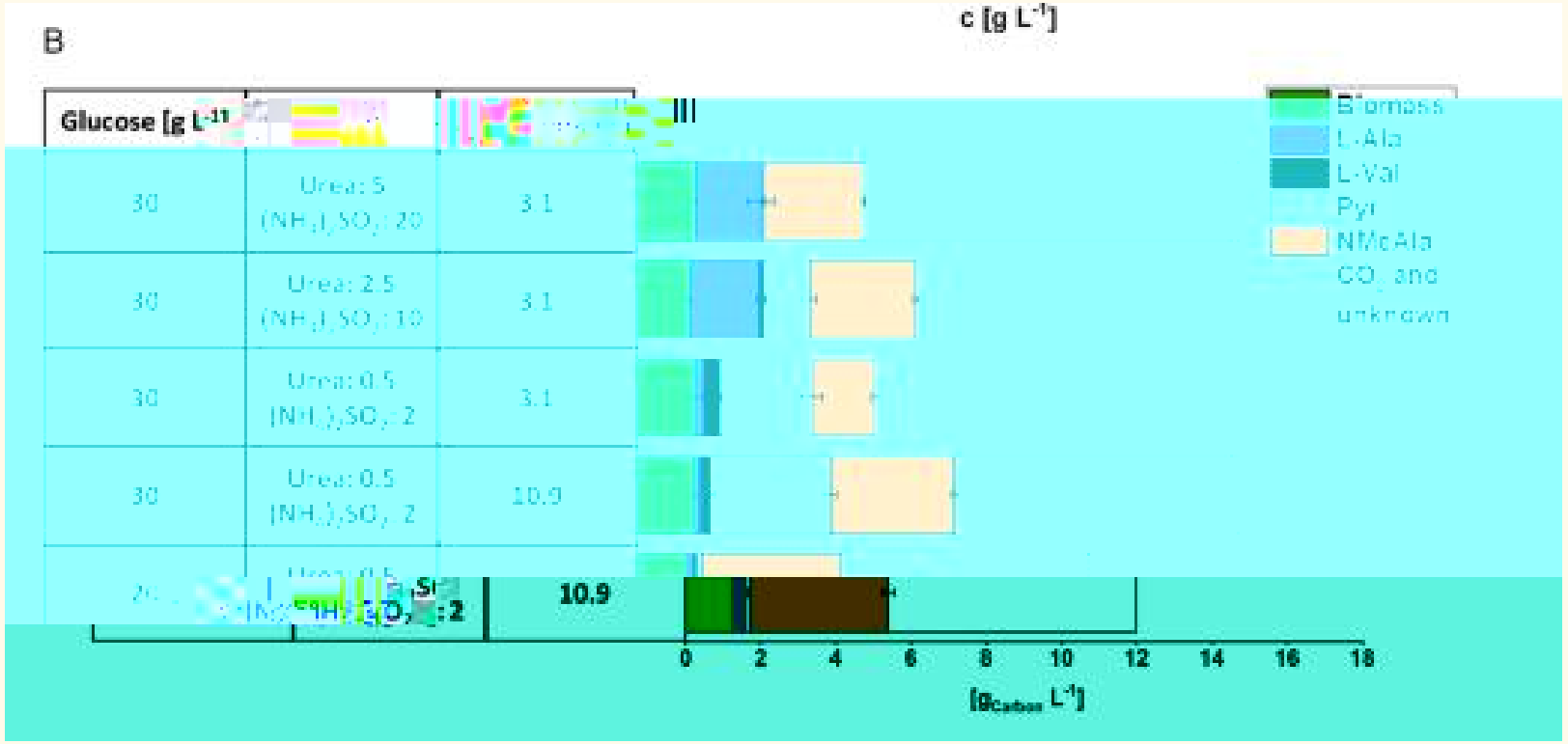
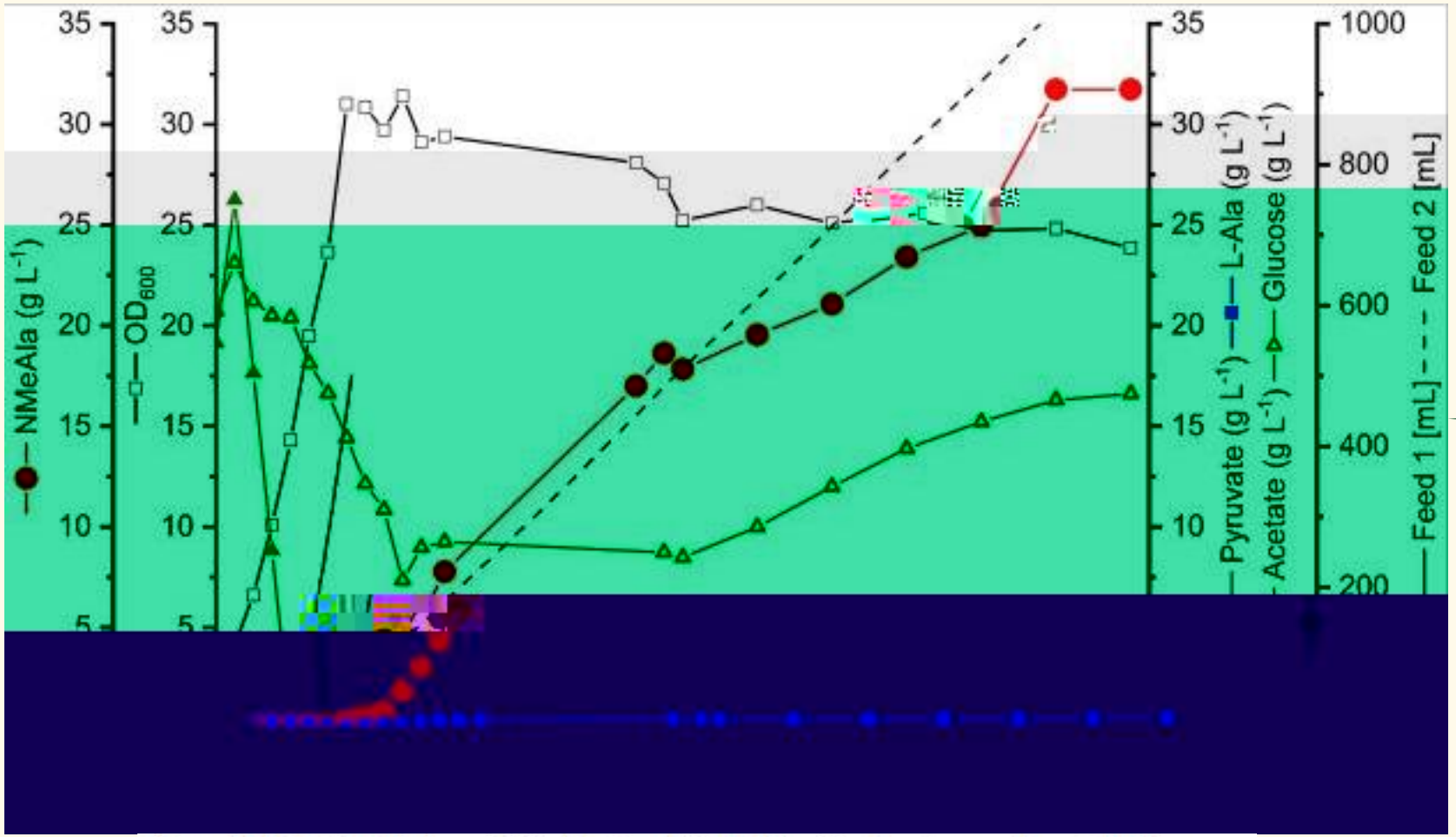

B
c [g L⁻¹]
Glucose [g L⁻¹]
30
Urea: 5
(NH4)2SO4: 20
3.1
30
Urea: 2.5
(NH4)2SO4: 10
3.1
30
Urea: 0.5
(NH4)2SO4: 2
3.1
30
Urea: 0.5
(NH4)2SO4: 2
10.9
10.9
Biomass
L-Ala
L-Val
Pyr
NMcAla
CO2 and unknown
0
2
4
6
8
10
12
14
16
18
[gCarbon L⁻¹]

A , 50% CO_2.

F -B NM A



NM A :

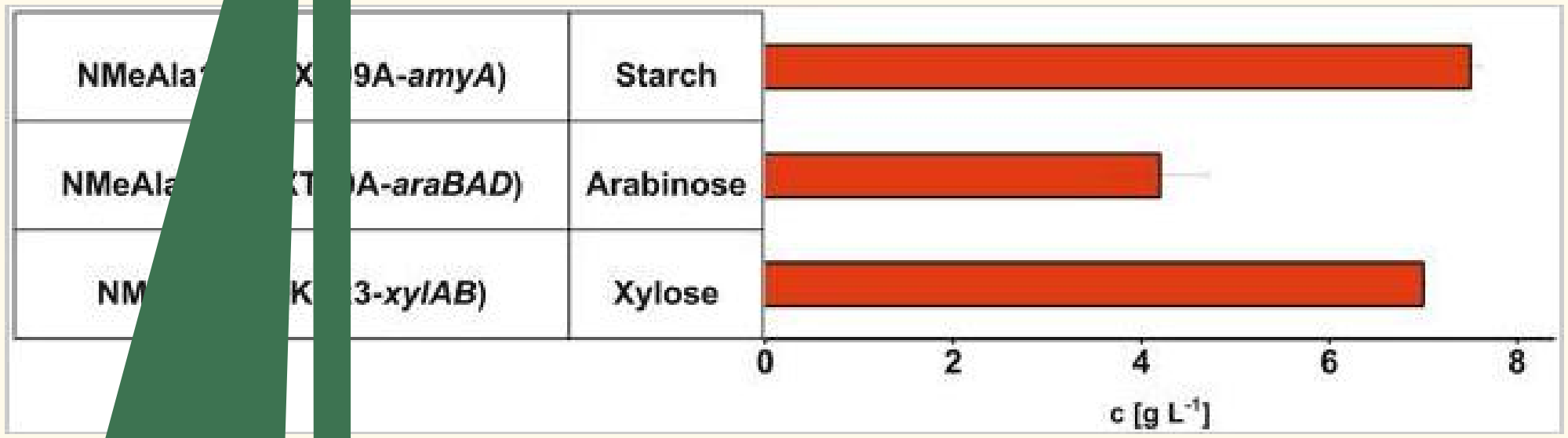
31.7 L 1

:

0.35 L 1 1

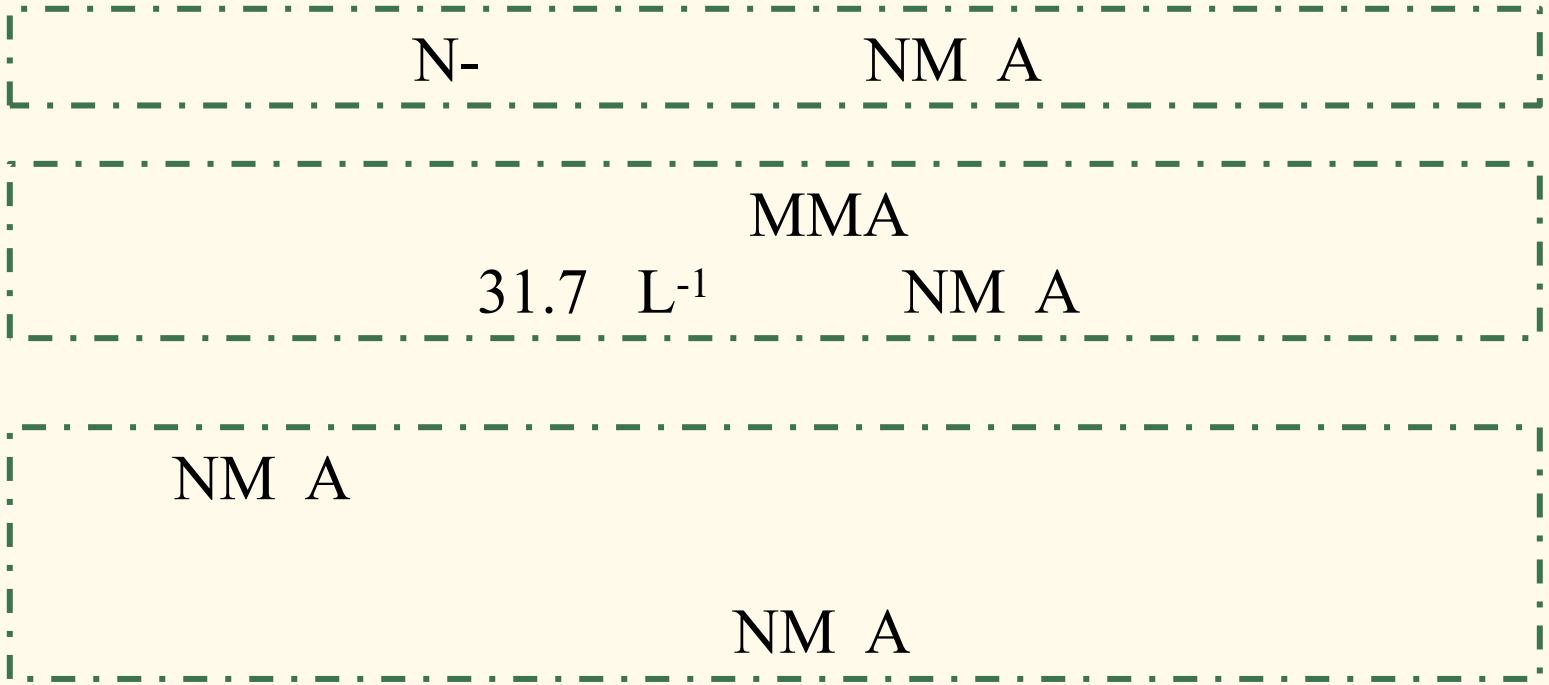
Figure 4. Fed-batch cultivation with *C. glutamicum* NMeAla1 in minimal medium supplemented with potassium acetate and glucose as carbon and energy sources. A fermenter with an initial start volume of 4 L was used. First feed phase (potassium acetate) was coupled to the rDOS value. After 22 h the second feed phase was started by the initial addition of 162 mL of a glucose/MMA solution followed by a linear feed of 12.3 mL h^{-1}. The biomass formation (black open squares), concentrations of NMeAla (red circles), L-alanine (blue squares), pyruvate (grey squares), acetate (green filled triangles) and glucose (green open triangles) were depicted. The volume of both feeds is shown as black lines. All depicted concentrations and the biomass formation was related to the initial volume.

E NM A E



Production of NMeAla from alternative carbon sources. The CGXII minimal medium with 16.6 g L^{-1} acetate contained 30 g L^{-1} starch for cultivation production experiments using *C. glutamicum* strain NMeAla1(pEKXT99A-*amyA*), 30 g L^{-1} arabinose using *C. glutamicum* strain NMeAla1(pEKXT99A-*araBAD*) and 30 g L^{-1} xylose using *C. glutamicum* strain NMeAla1(pEKEx3-*xylAB*). Concentrations were determined after 48 h and are given as means with standard deviations of three replicates.

C



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