

Tuning of the enzyme ratio in a neutral redox convergent cascade: A key approach for an efficient one-pot/two-step biocatalytic whole-cell system.

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Tables

TABLE 1. Plasmids Used in this Study

plasmid	Relevant characteristics	Replicon ^(a)	Reference
pET22b(+)	Amp ^R (MCS) T7 promoter	ColE1	Novagen
pETDuet-1	Amp ^R (MCS1 and MCS2) T7/lac promoter	ColE1	Novagen
pRSFDuet-1	Kan ^R (MCS1 and MCS2) T7/lac promoter	RSF1030	Novagen
pACYCDuet-1	Cm ^R (MCS1 and MCS2) T7/lac promoter	P15A	Novagen
pET22b- <i>bvmo</i>	pET22b LIC <i>bvmo</i> N term, 6histag	ColE1	Reignier <i>et al</i> , 2014
pET22b- <i>adh</i>	pET22b LIC <i>adh</i> N term, 6histag	ColE1	This work
pET- <i>adh</i>	pETDuet-1 <i>adh</i> (MCS1_MSC2 empty)	ColE1	This work
pET- <i>bvmo</i>	pETDuet-1 (MCS1 empty)_ <i>bvmo</i> (MCS2)	ColE1	This work
pET- <i>adh-bvmo</i>	pETDuet-1 <i>adh</i> (MCS1)_ <i>bvmo</i> (MCS2)	ColE1	This work
pRSF- <i>adh</i>	pRSFDuet-1 <i>adh</i> (MCS1_MCS2 empty)	RSF1030	This work
pRSF- <i>bvmo</i>	pRSFDuet-1 (MCS1 empty)_ <i>bvmo</i> (MCS2)	RSF1030	This work
pRSF- <i>adh-bvmo</i>	pRSFDuet-1 <i>adh</i> (MCS1)_ <i>bvmo</i> (MCS2)	RSF1030	This work
pACYC- <i>adh</i>	pACYCDuet-1 <i>adh</i> (MCS1_MCS2 empty)	P15A	This work
pACYC- <i>bvmo</i>	pACYCDuet-1 (MCS1 empty)_ <i>bvmo</i> (MCS2)	P15A	This work
pACYC- <i>adh-bvmo</i>	pACYCDuet-1 <i>adh</i> (MCS1)_ <i>bvmo</i> (MCS2)	P15A	This work

^(a) All the recombinant Duet vectors were constructed without histidine tag coding sequence (see Table S2).

TABLE 2. Description of the nine factors and the two level for Hadamard matrix

Level	Pre-culture media	Inoculum type	Initial OD ₆₀₀	Temperature before induction	Induction OD ₆₀₀	[IPTG] (mm)	Temperature after induction	Time after induction	Stirring (rpm)
+	<i>LB</i>	<i>LB agar</i>	0.4	37 °C	0.8	0.5	25 °C	48 h	180
-	TB	Cryotube	0.1	25 °C	0.6	0.2	16 °C	15 h	140

In ***bold*** and ***italics***, the values of the parameters set for the composite matrix.

TABLE 3. Strains constructed for co-expression on same (strains A, B and C) or different plasmids (strains D, E, F, G, H, I)

Strains	Plasmids
A	pRSF- <i>adh,bvmo</i>
B	pET- <i>adh,bvmo</i>
C	pACYC- <i>adh,bvmo</i>
D	pRSF- <i>bvmo</i> ; pET- <i>adh</i>
E	pET- <i>bvmo</i> ; pRSF- <i>adh</i>
F	pACYC- <i>bvmo</i> ; pET- <i>adh</i>
G	pRSF- <i>bvmo</i> ; pACYC- <i>adh</i>
H	pET- <i>bvmo</i> ; pACYC- <i>adh</i>
I	pACYC- <i>bvmo</i> ; pRSF- <i>adh</i>
Control	pET; pRSF