

ภาคผนวก ข
อาหารเลี้ยงเชื้อ

THIOBACILLUS MEDIUM (T)

KNO ₃	2.0	g
NH ₄ Cl	1.0	g
KH ₂ PO ₄	2.0	g
NaHCO ₃	2.0	g
MgSO ₄ .7H ₂ O	0.8	g
Na ₂ S ₂ O ₃ .5H ₂ O	5.0	g
Trace elements	1.0	ml
Distilled water to	1000	ml

Trace element solution:

Na ₂ -EDTA	50.0	g
ZnSO ₄ .7H ₂ O	2.2	g
CaCl ₂ .2H ₂ O	7.34	g
MnCl ₂ .4H ₂ O	2.5	g
FeSO ₄ .7H ₂ O	5.0	g
(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	0.5	g
CuSO ₄ .5H ₂ O	0.2	g
NaOH	11.0	g

Distilled water 1000.00 ml Adjust pH to 6.0 with KOH.

For storage, adjust pH to pH 4

For use the pH readjust to 6

ที่มา: <http://www.dsmz.de/dsmzhome.htm>

THIOBACILLUS THIOPARUS MEDIUM (TT MEDIUM)

$(\text{NH}_4)_2\text{SO}_4$	0.10	g
K_2HPO_4	4.00	g
KH_2PO_4	4.00	g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.10	g
CaCl_2	0.10	g
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0.02	g
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	0.02	g
$\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$	10.00	g
Distilled water	1000.0	ml
pH	6.6	

Reference: <http://www.dsmz.de/dsmzhome.htm>

THIOBACILLUS DENITRIFICANS MEDIUM

Solution A:

KH_2PO_4	2.0	g
KNO_3	2.0	g
NH_4Cl	1.0	g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.8	g
Trace element solution (see medium T)	2.0	ml
Distilled water	940	ml

Adjust pH to 7.0 with NaOH

Solution B:

$\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$	5.0	g
Distilled water 40.0	40.0	ml

Solution C:

NaHCO_3	1.0	g
Distilled water	20.0	ml

Solution D:

$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	2.0	g
H_2SO_4 (0.1N)	1.0	ml

Solutions A, B and D are separately sterilized by autoclaving at 121°C for 15 min. Solution C is sterilized by filtration or by autoclaving in a tightly closed vessel under an atmosphere of CO₂. After sterilization combine the four solutions and distribute as required under nitrogen atmosphere. For solid medium added 15 g agar to solution A.

Medium A (*Thiobacillus* sp. IW)^a

K ₂ HPO ₄	2	g
KH ₂ PO ₄	2	g
NH ₄ Cl	0.4	g
MgCl ₂ .6H ₂ O	0.2	g
FeSO ₄ .7H ₂ O	0.01	g
Na ₂ S ₂ O ₃ .5H ₂ O	8	g
Yeast extract	2	g
Distilled water	1000	ml
pH	7	

Reference: Park et al. 2002. Hydrogen sulfide removal utilizing immobilized *Thiobacillus* sp. IW with Ca-alginate bead. *Biochemical Engineering J.* 11, 167-173.

Medium B (A modification of thiosulfate medium ATCC290)

Na ₂ HPO ₄	2.27	g
KH ₂ PO ₄	1.8	g
MgCl ₂ .7H ₂ O	0.1	g
(NH ₄) ₂ SO ₄	1.98	g
MnCl ₂ .H ₂ O	0.023	g
CaCl ₂	0.03	g
FeCl ₃ .6H ₂ O	0.003	g
Na ₂ CO ₃	1	g
Na ₂ S ₂ O ₃ .5H ₂ O	15.69	g
Distilled water	1000	ml
pH	7	

Reference: Oyarzun, P. et al. 2003. Biofiltration of high concentration of hydrogen sulfide using *Thiobacillus thioparus*. *Process Biochemistry.* 39, 165-170.

อาหารสูตร C (*T. novellas*)

K_2HPO_4	4.0	g
KH_2PO_4	4.0	g
$MgSO_4$	0.8	g
Na_2EDTA	0.5	g
$ZnSO_4$	0.22	g
$CaCl_2$	0.05	g
$MnCl_2$	0.01	g
$FeSO_4$	0.001	g
$(NH_4)_6Mo_7O_{24}$	0.01	g
$CuSO_4$	0.01	g
$Na_2S_2O_3 \cdot 5H_2O$	10	g
Yeast extract	0.02	g
Distilled water	1000	ml
pH	7	

Reference: Cha et al. 1999. Removal of organosulphur odour compounds by *Thiobacillus novellus* SRM, sulphur-oxidizing microorganisms. Process Biochemistry. 34, 659-665.

สูตรอาหารสำหรับแยกเชื้อ purple nonsulfur photosynthetic bacteria

G-5 medium

peptone	0.5 g
Yeast extract	0.5 g
L-glutamic acid	0.4 g
DL-malic acid	0.35 g
KH_2PO_4	0.012 g
K_2HPO_4	0.018 g
Distilled water	1000 ml
pH	7

ปรับ pH ใช้ NaOH 5 N ก่อนนึ่งฆ่าเชื้อ ถ้าเป็นอาหารแข็งใช้วุ้น 1.5% บรรจุในหลอดทดลอง ปริมาณหลอดละ 5 ml นำไปนึ่งฆ่าเชื้อด้วยหม้อนึ่งความดันไอน้ำ 15 ปอนด์ต่อตารางนิ้ว อุณหภูมิ 121 องศาเซลเซียส เป็นเวลา 15 นาที

Medium for denitrification process

Nitrate broth

Beef extract	3.0 g
Peptone	5.0 g
Potassium nitrate	1.0 g
Distilled water	1000 ml

ละลายส่วนผสมทั้งหมดลงในน้ำโดยใช้ความร้อนช่วยบรรจุหลอดทดสอบซึ่งมี Durham tube นำไปนึ่งฆ่าเชื้อด้วยหม้อนึ่งความดันไอน้ำ 15 ปอนด์ต่อตารางนิ้ว อุณหภูมิ 121 องศาเซลเซียส เป็นเวลา 15 นาที

Protein hydrolysis

Casein medium

Tryptone	5.0 g
Yeast extract	2.5 g
Glucose	1.0 g
Agar	15.0 g
Skim milk	20 ml
Distilled water	980 ml

ใส่ Skim milk ก่อนผสมลงในขวด นำไปนึ่งฆ่าเชื้อด้วยหม้อนึ่งความดันไอน้ำ 15 ปอนด์ต่อตารางนิ้ว อุณหภูมิ 121 องศาเซลเซียส เป็นเวลา 10 นาที แล้วทำให้เย็นทันที

การนำเสนอผลงาน

ส่วนหนึ่งของงานวิจัยนี้ได้นำเสนอแบบโปสเตอร์ในการประชุมสัมมนา

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Screening of *Thiobacillus* sp. for their Ability to Remove Sulfide from Rubber Wastewaters

Duangporn Kantachote¹, Wilawan Charernjiratrakul¹, Napavarn

Noparatnaraporn² and Kohei Oda³

¹*Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat-Yai, Thailand*

²*Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok*

³*Department of Applied Biology, Faculty of Textile Science, Kyoto Institute of Technology, Kyoto, Japan*

In order to remove sulfide in rubber wastewaters, a total of 147 isolates of *Thiobacillus* sp. were isolated from various sources. This included 69 mesophiles (30°C) and 78 thermophile (50°C). Only 8 of the mesophilic isolates and 14 of the thermophiles grew well in sterile untreated rubber wastewater in both a sulfate reducing reactor (SRR) and an up-flow anaerobic sludge blanket (UASB). These were selected for further study. However, only the thermophilic species showed promise for the removal of sulfide. *Thiobacillus* sp. TT502 gave the best sulfide reduction in the SRR (initial concentrations of 118mg/L total sulfide, 93mg/L dissolved sulfide and 2.01mg/L unionized hydrogen sulfide: UHS) removing 81% of the total sulfide, 84% of dissolved sulfide and 72% of UHS. In the UASB, the isolate TT5036 removed 68% of total sulfide, 75% of dissolved sulfide and 89% of UHS starting with concentrations of 88mg/L total sulfide, 80mg/L dissolved sulfide and 1.49mg/L UHS. Interestingly, most of the selected isolates had proteolytic activity that was higher with gelatin than with casein.

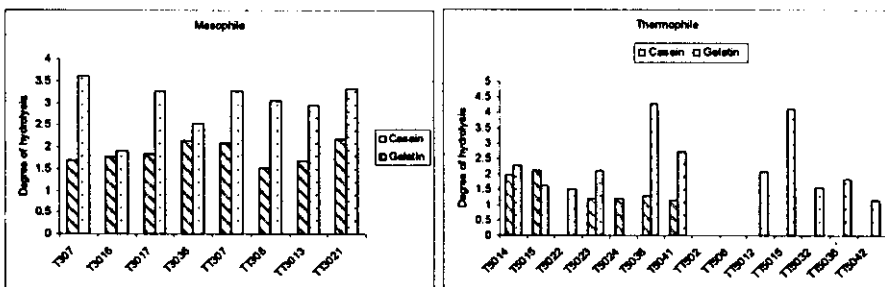


Figure Proteolytic activity of the selected isolates of *Thiobacillus* sp.