Production of 5-Aminolevulinic Acid from Monosodium Glutamate Effluent by Halotolerant Photosynthetic Bacterium (*Rhodobacter capsulatus* SS3)

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Abstract: Thai people consume high quantity of monosodium glutamate in which its production process generates large amount of effluent. Monosodium glutamate effluent (MSGE) has the following average values; pH 3.59, 220,100 mg/l COD, 0.814 g/l reducing sugar, 3.72 % v/v total nitrogen, 6.18 g/l NaCl, and 11.56 % w/w glutamic acid. The organic matter content (COD value) of MSGE was about 2 and 3 folds higher than those of distillery stillage (DS) (115,600 mg/l COD), and synthetic medium (GSY) (7,740 mg/l COD), respectively. These three media were used for growth and 5-aminolevulinic acid (ALA) production from halotolerant photosynthetic bacteria, *Rhodobacter capsulatus* SS3, by cultivating under aerobic-dark condition (150 rpm agitation rate) at 37°C for 4 days. Results revealed that good growth was observed in GSY and MSGE, but not in DS, giving the dry cell weight (DCW) of 4.29 g/l and 3.70 g/l, respectively after 36 h cultivation. However, the strain produced high concentration of ALA (40 μM) in GSY medium whereas very low amount of ALA (0.20 μM) was found in MSGE medium. To improve ALA production, MSGE was diluted to give the COD values of 3,426, 7,644, and 15,288 mg/l and NaCl concentration was adjusted to the level present in GSY medium. The ALA production was highest using GSY medium followed by using MSGE with COD of 15,288 mg/l (18.6 μM). Further increase of ALA concentration to 24 μM in MSGE was achieved by supplementation with organic acids, glycine, and mineral. This resulted in 120 folds higher than MSGE (0.2 μM).

Keywords : 5-Aminolevulinic Acid (ALA), Photosynthetic Bacteria, Monosodium Glutamate Effluent (MSGE), Glucose-salt-yeast Extract Medium (GSY).

1. INTRODUCTION

Agro-industry plays a significant role in the economic growth of Thailand while being the source of high polluting wastewater, for example, monosodium glutamate (MSG) effluent and distillery stillage (distillage). MSG is the sodium salt of amino acid, glutamic acid, and is widely used as flavor enhancer. It is generally produced by microorganism using sugar beets or cassava as carbon source and its production process generates large amount of effluent. MSG effluent, often warm, has high salt content and has the average values as following; 14,000 mg/l COD, 0.485 g/l glutamic acid, 3.72 % total nitrogen and pH 3.6. Experiment at Zhangjiagang MSG Plant in China for over a year demonstrated that the MSG effluent could be treated to contain less than 1,000 mg/l COD, <100 mg/l ammonia nitrogen and <2,000 mg/l sulphate. Besides MSG effluent, distillate has the following average values (mg/l); BOD 27,850, COD 184,000, reducing sugar 35,200, solid 2,345, nitrogen 2,700 and average temperature of 92 °C with pH 4.4 (Prasertsan and Suksawat, 1982). Since distillate contains many yeast cells that can be the source of vitamins, it is considered to be the nourishing source for bacterial growth.

Alternative approach for treatment of these wastewater is to use as substrate for growth of particular microorganisms which can produce valuable products. Photosynthetic bacteria is proposed to be the appropriate microorganism as it produces 5-aminolevulinic acid (ALA), which is used as herbicide, insecticide, growth stimulator, etc. It was reported that ALA from *Rhodobacter sphaeroides* can be produced in the effluent of swine waste from an anaerobic digestor and enhanced by adding glycine (20 mM) plus levulinic acid (5-60 mM) (Sasaki *et al.*, 1990). Besides environmental aspect, ALA could also be applied to medical field in photodynamic therapy for cancer treatment. The research work aims to utilize and treat agro-industrial wastes using photosynthetic bacteria to produce valuable product especially 5-aminolevulenic acid (ALA) and apply ALA as bioherbicide and growth promoting factor.

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2. MATERIAL AND METHODS

2.1 Microorganism

The halotolerant photosynthetic bacterium, *Rhodobacter capsulatus* SS3, isolated by Tangprasittiparp (1999), was used in this study. The strain was maintained on agar slant of glutamate-malate (GM) medium (Lascelles, 1956) and kept at the culture collection of the Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University.

2.2 Medium

2.2.1 Glucose-salt-yeast extract (GSY) medium

GSY medium contained 50 mM glucose and 1.5 g/l yeast extract with 30 g/l NaCl (Madmarn, 2002). The pH was adjusted to 6.5.

2.2.2 Cultivation medium

Agro-industrial wastewater used in this study were monosodium glutamate effluent (MSGE) and distillery stillage

(DS) which were obtained from Thai Fermentation Industry Co., Ltd., Rajburi province and Nateechai Co., Ltd., Suratthanee province, respectively. The supernatant (by centrifugation with 10,000 rpm for 5 min) of both wastewaters were used and adjusted pH to 6.5.

2.3 Analytical methods

2.3.1 Dry cell weight

Cells are centrifuged at 10000 rpm for 15 min, rinsed by distilled water twice and dried at 103 °C overnight or until constant weight is obtained (Sattayasmithstid, 2002).

2.3.2 Extracellular ALA

Extracellular ALA is determined by colorimetric method (Sasaki *et al*., 1987). The supernatant of the cell culture is transferred to 2 ml of acetate buffer (1 M, pH 4.7) containing 50 µl of acetylacetone. The solution is heated in boiling water for 15 min, then quickly cool and react with 3.5 ml of Ehrlich's reagent, visualizing purple-red color in 15 minutes. The extracellular ALA is measured with spectrophotometer (OD_{553}).

2.4 Method

2.4.1 Formulation and selection of production media from monosodium glutamate effluent (MSGE) and distillery stillage (DS)

2.4.1.1 Determination of chemical composition of waste media

MSGE, DS and GSY media were analyzed for COD (APHA, AWWA, and WPCF, 1985), nitrogen (AOAC, 1984), reducing sugar (Nelson, 1954), glutamic acid (analyze by High Performance Liquid Chromatography method at Scientific Equipment Center, Mahidol University) and NaCl (analyze by Inductively Coupled Plasma Optical Emisssion Spectrometry method at Scientific Equipment Center, Prince of Songkla University).

2.4.1.2 Comparison on growth, extracellular ALA production and COD removal of *R.capsulatus* SS3 cultivating in wastewater and synthetic medium

Cultivation of *R.capsulatus* SS3 was conducted in MSGE, DS, and GSY medium and compared the bacterial growth and extracellular ALA production.

2.4.1.3 Formulation of waste media (formulated waste media)

Formulation of waste media from these two sources of agro-industrial wastes were conducted based on the same concentration of COD, nitrogen, and NaCl as those of GSY medium with the distilled-water addition, if necessary, of molasses, NH_4NO_3 and NaCl respectively.

2.4.2 Comparison on growth and ALA production of photosynthetic bacteria cultivating in the waste and formulated waste media

2.4.2.1 Inoculum preparation

One loop of *Rhodobacter capsulatus* SS3 from GSY agar slant was inoculated into 100 ml of GSY medium and cultivated under aerobic-dark condition (150 rpm) at 37 °C for 24 h. Turbidity is measured by spectrophotometer at 660 nm wavelength and adjusted to 0.5 using GSY medium before using as the inoculum.

2.4.2.2 Cultivation in formulated waste media

Starter (10%) of *Rhodobacter capsulatus* SS3 in GSY medium was added into 100 ml of flask containing GSY, and formulated media (MSGE and DS media) and cultivated for 4 days under aerobic-dark condition (150 rpm) in incubated shaker at 37 °C. Samples (10 ml) were taken every 6 h to measure for pH, growth (OD_{660}), extracellular ALA. The formulated waste medium giving the highest ALA concentration was selected for further studies.

2.4.2.3 Effect of waste source (based on equal COD and nitrogen concentration of GSY medium), COD concentration, total nitrogen concentration, and sodium content of the medium

The effect of COD concentration, total nitrogen concentration and sodium content of wastewater (MSGE and DS) and formulated waste medium were studied. Cultivation was conducted as describe above and compared the bacteria growth/ ALA production on the selected medium with different concentrations of COD, nitrogen and sodium content.

2.4.2.4 Effect of organic acid, glycine, mineral, and levulinic acid (LA) added in formulated medium

To improve the production of ALA from wastes, supplementation of 10 mM glycine, 40 mM succinic acid, 0.5 g/l propionic acid, 15 mM $MgCl_2$, and adding 15 mM levulinic acid (LA) at 18 h in synthetic medium, waste and formulated waste media. Results were compared with those media without supplemementation.

3. RESULTS AND DISSCUSSIONS

3.1 Formulation of waste media from monosodium glutamate effluent (MSGE) and distillery stillage (DS)

3.1.1 Determination of chemical composition of waste media

Glucose-salt-yeast extract medium (GSY) was the synthetic medium for growth of *Rhodobacter capsulatus* SS3 and *Rhodobacter sphaeroides* ES16 (Madmarn, 2002). Comparison on the characteristics of GSY medium with those of monosodium glutamate effluent (MSGE) and distillery stillage (DS) (Table 1) indicated that MSGE had higher concentrations of COD (220,100 mg/l), reducing sugar (RS) (813.62 ug/ml), total nitrogen (3.72 %v/v), and sodium content (6.18 g/l) than DS (115,600 mg/l COD, 370.94 µg/ml RS, 0.99 % nitrogen, and 0.57 g/l sodium content). In addition, MSGE also contained 11.56 % w/w glutamic acid. Both wastewaters were more acidic (pH 3.60 and 4.24, respectively) than the GSY medium (pH 6.5).

Table 1 Characteristics of monosodium glutamate effluent (MSGE), distillery stillage (DS) and glucose-salt-yeast extract medium (GSY)

Parameter	GSY	MSGE	DS
pH	6.5 ± 0.1	3.60 ± 0.1	4.24 ± 0.1
COD (mg/l)	7,740 ± 94.0	220,100 ± 206.5	115,600 ± 128.7
Reducing sugar (μg/ml)	121.32 ± 0.27	813.62 ± 0.21	370.94 ± 0.05
Total nitrogen (%)	0.08	3.72 ± 0.001	0.99 ± 0.001
Sodium content (g/l)	30	6.18 ± 0.08 *	0.57 ± 0.04 *
Glutamic acid content (% w/w)	-	11.56 **	-

* Analysed at Scientific Equipment Center, Prince of Songkla University

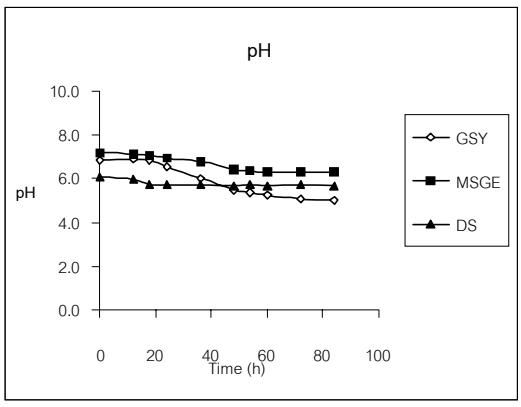
**Analysed at Scientific Equipment Center, Mahidol University

\- Not determined

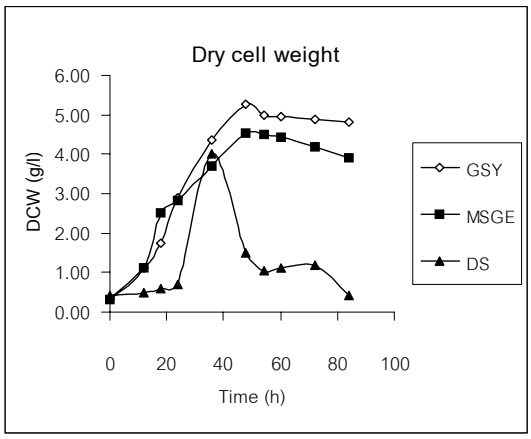
3.1.2 Comparison on growth, extracellular ALA production and COD removal of photosynthetic bacterium (*Rhodobacter capsulatus* SS3) cultivating in wastewater and synthetic medium

Cultivation of *Rhodobacter capsulatus* SS3 in MSGE, DS, and GSY medium for extracellular ALA production was conducted (Fig. 1).

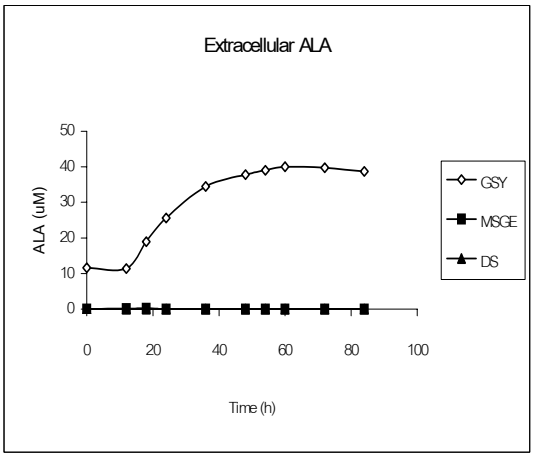
During cultivation in all media, the pH values decreased from 6.86, 7.20, and 6.07 to 5.03, 6.31, and 5.68, respectively. The strain grew better in GSY medium than in MSGE and DS, giving the dry cell weights of 5.26, 4.55, and 4.00 g/l, respectively. Extracellular ALA production was highest (40.05 μM) in GSY medium while only small amount (0.2 μM) was produced in MSGE and not produced in DS. MSGE was a better source than DS as it contained higher organic matter, in term of COD values especially glutamic acid. The consumption of the organic substances resulting in the COD removal 29.58 % in MSGE which was higher than that in DS (20.16 %) but lower than in GSY (54.76 %) (Table 2). Between these two sources of wastewater, MSGE was selected for further studies.



(a) pH



(b) Dry cell weight



(c) Extracellular ALA

Fig. 1 Effect of different medium on pH (a), dry cell weight (b), and extracellular ALA production (c) during cultivation of *Rhodobacter capsulatus* SS3 under aerobic-dark condition on a shaker (150 rpm) at 37 °C for 4 days.

Table 2 COD removal of GSY, MSGE, and DS

COD removal	GSY	MSGE	DS
- Initial	7,740	220,100	115,600
- Final	3,501	155,000	92,300
% COD removal	54.76	29.58	20.16

3.1.3 Formulation of waste media (formulated waste media)

The two sources of agro-industrial wastewaters were formulated for production of ALA based on the same concentration of COD, nitrogen, and NaCl as those of GSY medium in six formula were given as following :

A : MSGE (23.5 folds dilution) with COD value of 7,644 mg/l, COD of GSY medium was 7,740 mg/l

B : MSGE (46.5 folds dilution) with total nitrogen value of 0.07 %v/v, total nitrogen of GSY medium was 0.08 %v/v

C : MSGE (add 23.82 g/l NaCl) with sodium content value of 30 g/l, sodium content of GSY medium was 30 g/l

D : DS (14.9 folds dilution) with COD value of 7,620 mg/l, COD of GSY medium was 7,740 mg/l

E : DS (12.4 folds dilution) with total nitrogen value of

0.07 %v/v, total nitrogen of GSY medium was 0.08 %v/v
F : DS (add 29.43 g/l NaCl) with sodium content value of 30 g/l, sodium content of GSY medium was 30 g/l.

3.2 Factors affecting ALA production from waste and formulated waste media of photosynthetic bacterium cultivating

The effects of COD concentration, total nitrogen concentration, and sodium content of GSY, MSGE, and DS were studied (Fig. 2, 3, 4). Cultivation of *R. capsulatus* SS3 in GSY medium as well as formulated MSGE and DS based on the same concentration of COD (formula A and D, respectively), nitrogen (formula B and E, respectively), and NaCl (formula C and F, respectively) as those of GSY medium. The pH values of GSY, formula C, D, E, and F decreased from 6.57, 7.35, 6.17, 6.06, and 6.06 to 5.41, 6.40, 5.49, 5.54, and 5.93, respectively whereas the pH values of formula A and B increased from 6.99 and 7.04 to 8.41 and 8.22, respectively (Fig. 2a).

3.2.1 Effect of waste source based on equal COD and nitrogen concentration of GSY medium

From the result**,** Formula A gave higher concentrations of dry cell weights (0.9762 g/l) and extracellular ALA (8.3258 μM) than formula D (0.4481 g/l and 0 μM, respectively) (Fig. 3) and

R. capsulatus SS3 grew better in formula B than in formula E, giving higher concentrations of dry cell weights (0.6237 g/l) and extracellular ALA (5.3371 μM) than formula E (0.3500 g/l, 0 μM, respectively) (Fig. 3).

Thus the best waste source was formula A; MSGE which based on the same COD concentration of GSY medium since it gave highest dry cell weight and ALA production (0.9762 g/l and 8.3258 μM, respectively).

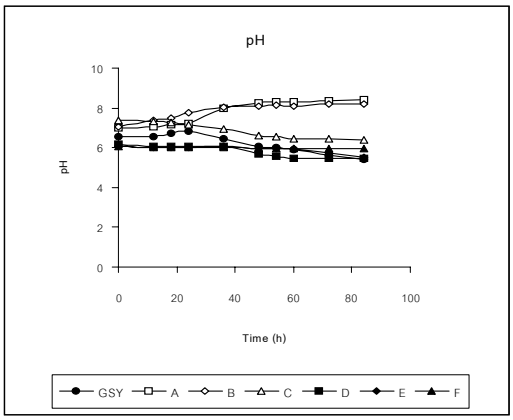
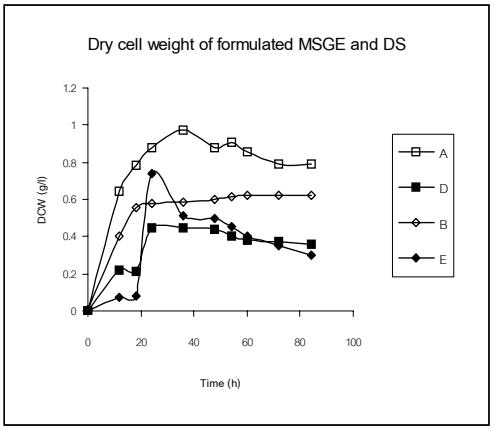


Fig. 2 pH from cultivation of *Rhodobacter capsulatus* SS3 in GSY and formulated MSGE (A-C) and DS (D-F) under aerobic-dark condition on a shaker (150 rpm) at 37 °C for 4 days.



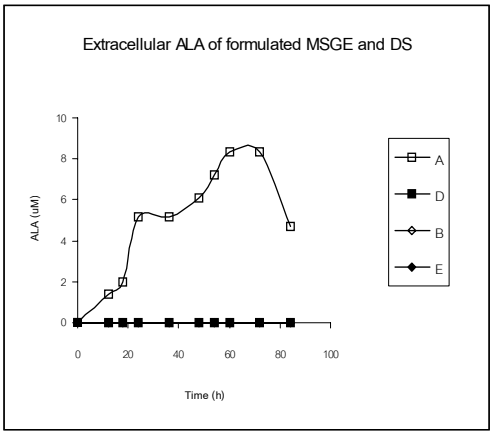
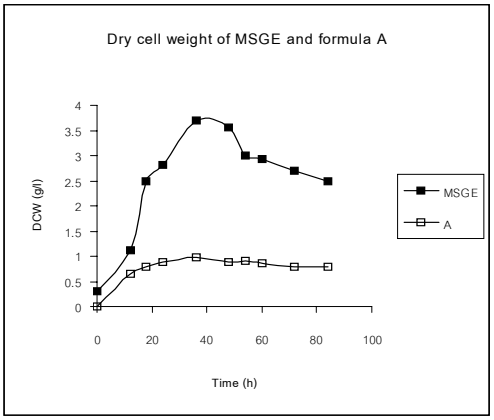


Fig. 3 Dry cell weight and extracellular ALA production from *R. capsulatus* SS3 cultivating in waste based on equal COD (formula A, D) and nitrogen concentration (formula B, E) of GSY medium.

3.2.2 Effect of COD concentration

R. capsulatus SS3 grew better in formula A than MSGE. Formula A gave higher concentrations of dry cell weights (0.9762 g/l) and extracellular ALA (8.3258 μM) than MSGE (0.4481 g/l and 0 μM, respectively) (Fig. 4).



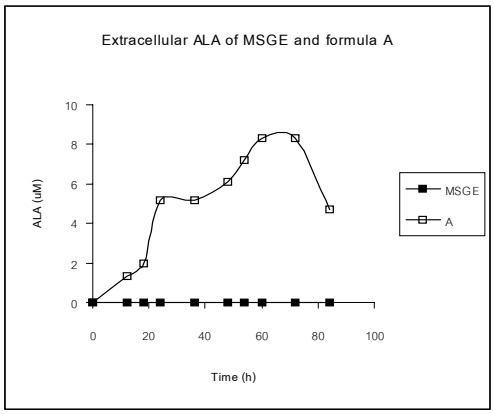
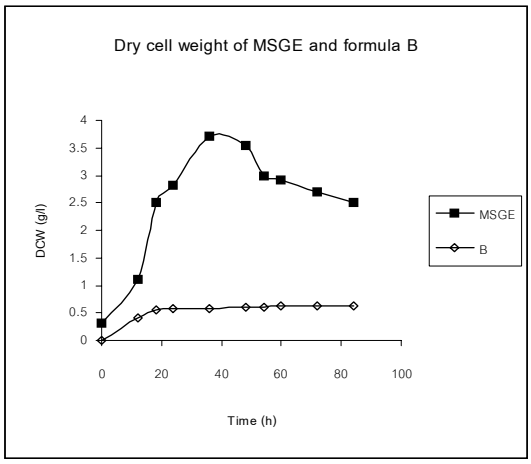


Fig. 4 Effect of COD concentration of MSGE and formula A from *Rhodobacter capsulatus* SS3 cultivation under aerobic-dark condition on a shaker (150 rpm) at 37 °C for 4 days.

3.2.3 Effect of nitrogen concentration

R. capsulatus SS3 grew better in formula B than MSGE. Formula B giving higher concentrations of dry cell weights (0.6237 g/l) and extracellular ALA (5.3371 μM) than MSGE (0.3500 g/l, 0 (M, respectively) (Fig. 5).



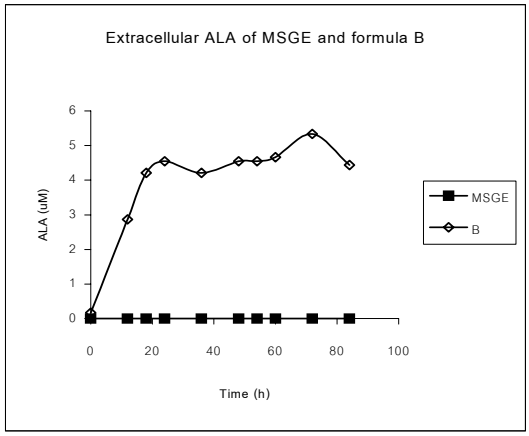
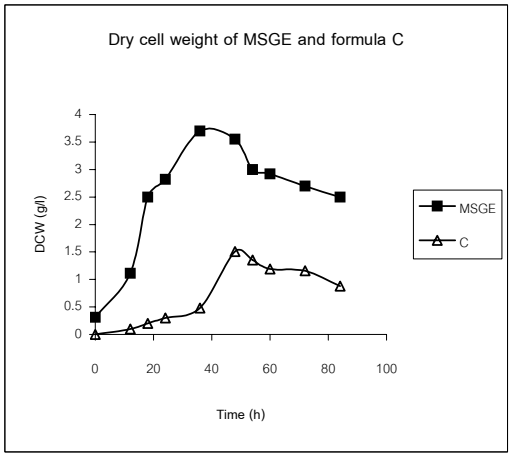


Fig. 5 Effect of nitrogen concentration of MSGE and formula B from *Rhodobacter capsulatus* SS3 cultivation under aerobic-dark condition on a shaker (150 rpm) at 37 °C for 4 days.

3.2.4 Effect of NaCl concentration

Both MSGE and formula C did not produce extracellular ALA (Fig. 6).

Extracellular ALA production from *R. capsulatus* SS3 was highest (40 M) in GSY (C/N ratio = 9,675), followed by MSGE (formula A, C/N ratio = 47,775) (8.3258 M) and MSGE (formula B, C/N ratio = 54,600) (5.3371 M), respectively (Fig. 2c). Thus the optimum C/N ratio for R. capsulatus SS3 cultivation in formulated waste media was between 9,675 to 54,600.



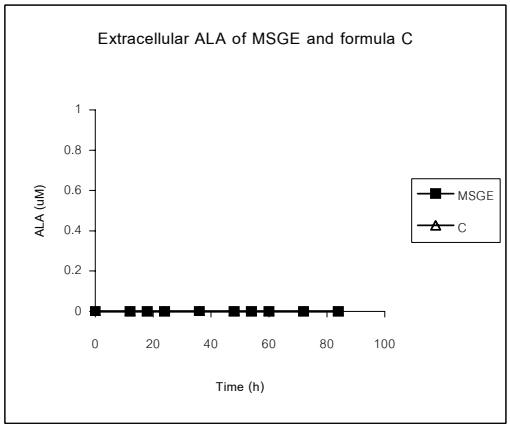


Fig. 6 Effect of sodium content in MSGE and formula C from *Rhodobacter capsulatus* SS3 cultivation under aerobic-dark condition on a shaker (150 rpm) at 37 °C for 4 days.

3.2.5 Effect of supplementation with nutrient in formulated MSGE

Since formulated waste media gave very low ALA production, supplementation with nutrients, as given below, were studied and results were illustrated in Fig. 7, 8, 9.

Sample X	Sample Y *
G. GSY	M. GSY
H. MSGE	N. MSGE
I. MSGE (46.5 folds dilution)	O. MSGE (46.5 folds dilution)
J. MSGE (23.5 folds dilution)	P. MSGE (23.5 folds dilution)
K. MSGE (12 folds dilution)	Q. MSGE (12 folds dilution)
L. MSGE (add NaCl 23.82 g/l)	R. MSGE (add NaCl 23.82 g/l)

* The supplementation in sample B contains 10 mM glycine, 40 mM succinic acid, 0.5 g/l propionic acid, 15 mM $MgCl_2$, and added 15 mM levulinic acid (LA) at 18 h (Sattayasmithstid, 2002).

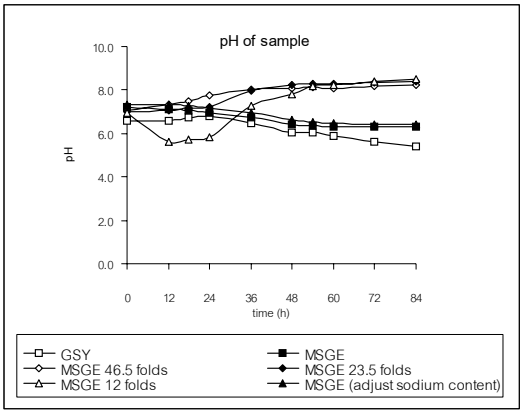
I and O; MSGE (46.5 folds dilution) with COD value of 3,426 mg/l and had total nitrogen about 0.07 % that it was adjusted total nitrogen as similar as total nitrogen in GSY (total nitrogen = 0.08 %)

J and P; MSGE (23.5 folds dilution) with COD value of 7,644 mg/l, COD of GSY medium was 7,740 mg/l

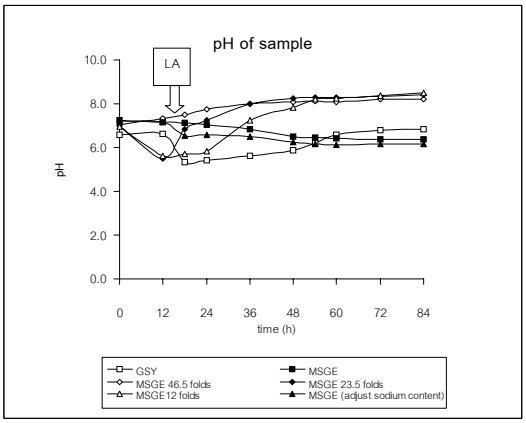
K and Q; MSGE (12 folds dilution) with COD value of 15,288 mg/l

L and R; MSGE (added NaCl 23.82 g/l) with sodium content of 30 g/l, sodium content of GSY medium was 30 g/l

3.2.5.1 pH of R. capsulatus SS3 cultivation in GSY and formulated MSGE



(X)

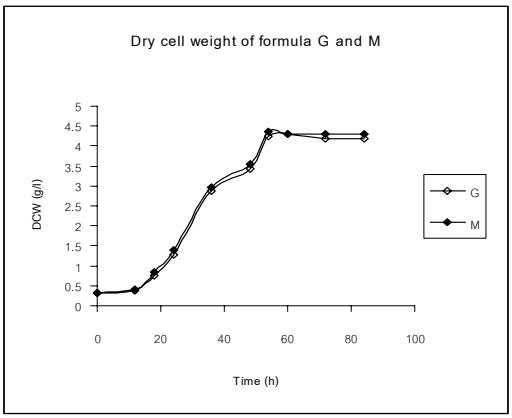


(Y)

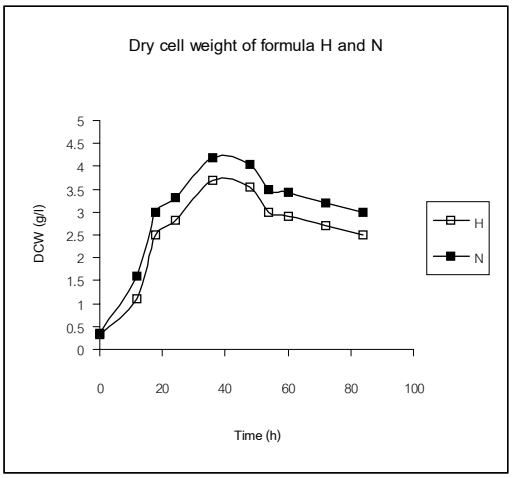
Fig. 7 pH changes of *R. capsulatus* SS3 cultivation in each media (X) and media with the nutrients supplementation (Y) under aerobic-dark condition on a shaker (150 rpm) at 37 °C for 4 days.

In Fig. 7 the pH of this cultivation was between 6 and 8.5 and pH of formulated MSGE was higher than GSY. Fig. 7(Y), pH of MSGE (nutrients supplementation) and GSY (nutrients supplemena\tation) was rapidly decreased at 18 h cultivation due to the addition of 15 mM levulinic acid (LA) ; in after pH of MSGE and GSY increased to normal pH (about 6.5) since LA inhibited ALA dehydratase at that time.

3.2.5.2 Dry cell weight of *R. capsulatus* SS3 cultivation in GSY and formulated MSGE



(a)



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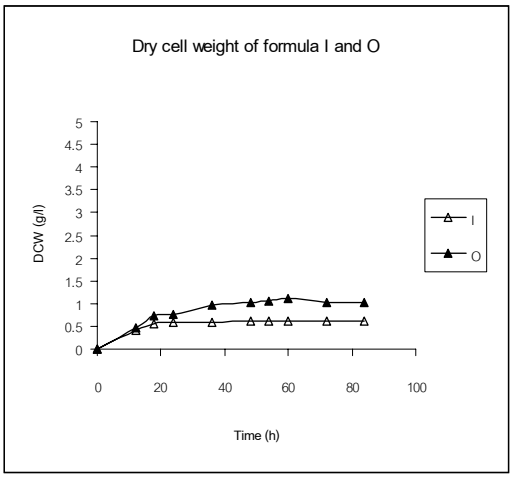

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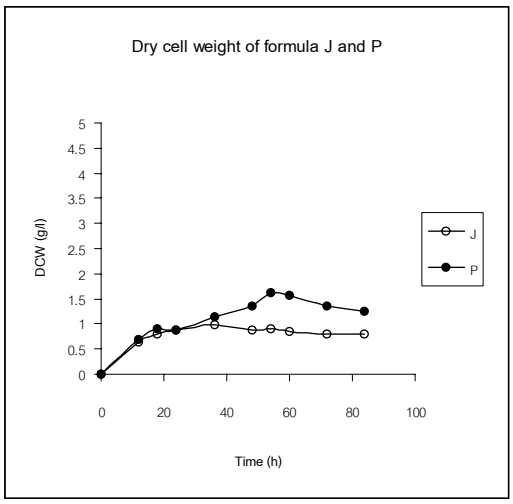
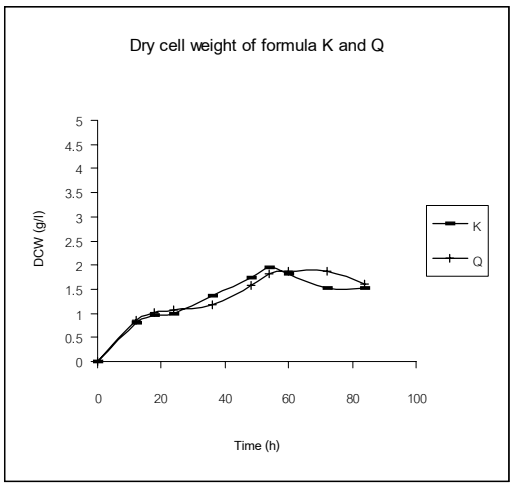
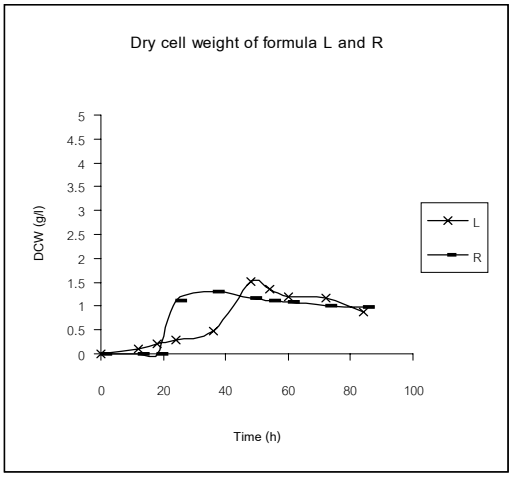


(d)



(e)

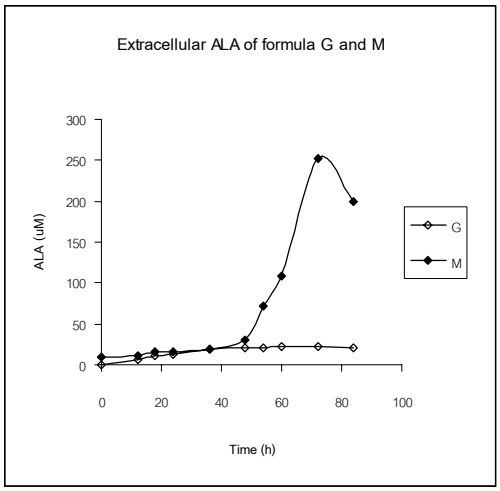


(f)

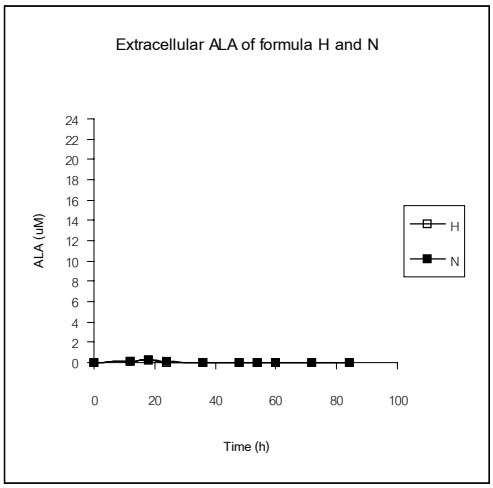
Fig. 8 Dry cell weight of *R. capsulatus* SS3 cultivation in (a) synthetic media (formula G and M), (b) waste media (formula H and N), and (c, d, e, f) formulated waste media with (formula I-L) and without nutrients supplementation (formula O-R) under aerobic-dark condition on a shaker (150 rpm) at 37 °C for 4 days.

Dry cell weight values were between 0 and 5 g/l, with DCW in formula G (4.29 g/l) was higher than formula K(diluted 12 folds; 1.96 g/l) and dry cell weight of formula H (3.70 g/l) was higher than formula J and I (diluted 23.5 folds, 46.5 folds in 0.91 g/l and 0.62 g/l, respectively) since MSGE may be contained appropriate nutrient than the formulated MSGE in dilution 23.5 folds and 46.5 folds, respectively. Figure 8; DCW of formula K (12 folds diluted; 1.96 g/l) was higher than another formulated waste media (formula I, J, L, and O-R), as well as nutrients supplementation stimulated growth of *R. capsulatus* SS3 and induced ALA quantity (Sattayasmithstid, 2002). It showed that MSGE (12 folds diluted) with organic acid, glycine, and mineral addition was the production media for extracellular ALA formation (Fig. 9) with the lag phase in the first 24 h., log phase was 24-60 h., and stationary phase was 60-72 h. On the other hand, formula G, H, I, J gave dry cell weight of 4.29 g/l and 3.70 g/l, 0.62 g/l and 0.91 g/l, respectively) which less than formula M, N, O, P (4.36 g/l, 4.05 g/l, 1.11 g/l, and 1.63 g/l, respectively) since the essential organic compounds were used to produce ALA rather than to activate *R. capsulatus* SS3 growth (ALA production rate; q_p value (h^{-1}) was higher than bacterial growth rate; dry cell weight (g/l)). Whereas Fig. 8 and 9; nutrients supplementation in formula Q were important for ALA production than *R. capsulatus* SS3 growth that it could be produced extracellular ALA in high values (23.74 μM) but they gave dry cell weight in low values (1.87 g/l). And dry cell weight in formula R (1.30 g/l) was lower than formula L (1.51 g/l) that may be sodium chloride addition inhibited nutrients supplementation for *R. capsulatus* SS3 growth.

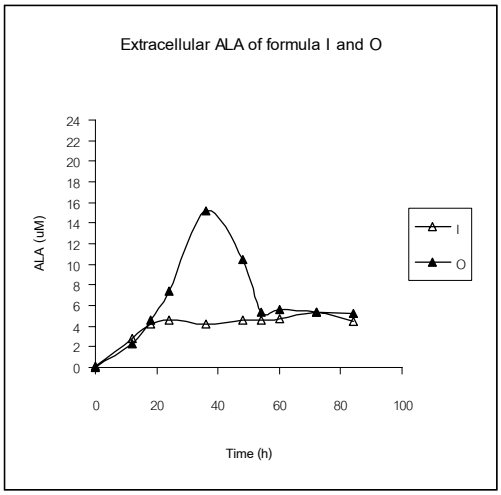
3.2.5.3 Extracellular 5-aminolevulinic acid of *R. capsulatus* SS3 cultivation in GSY and formulated MSGE



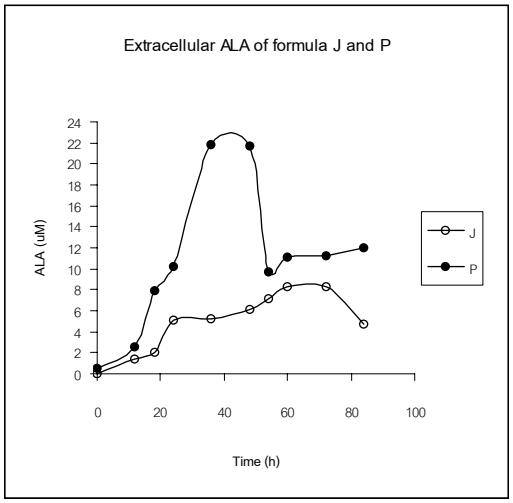
(a)



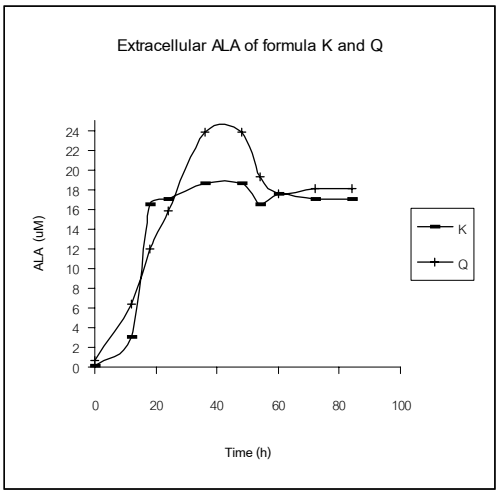
(b)



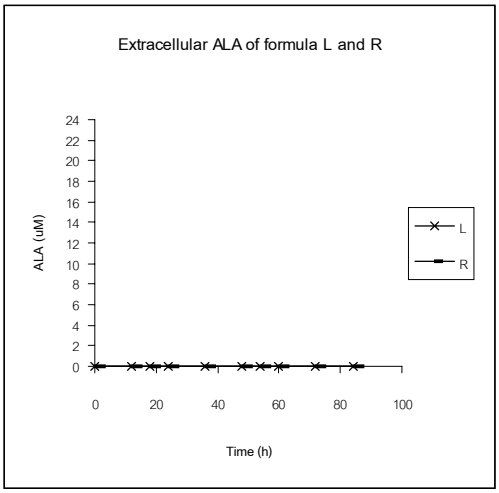
(c)



(d)



(e)



(f)

Fig. 9 Extracellular ALA of *R. capsulatus* SS3 cultivation in (a) synthetic media (formula G and M), (b) waste media (formula H and N), and (c, d, e, f) formulated waste media with

(formula I-L) and without nutrients supplementation (formula O-R) under aerobic-dark condition on a shaker (150 rpm) at 37 °C for 4 days.

From Fig. 9, ALA production of this cultivation was between 0 and 300 μM in formulated GSY (formula M) that ALA production in formula M was higher than formula G and formula H-L, N-R (formulated MSGE in 46.5, 23.5, 12 folds dilution). Fig. 9; ALA production of formula M (252 μM) was higher than formula Q (supplementation & diluted 12 folds; 24 μM) and ALA in formula M (252 μM) was higher than formula G (40 μM) in 6.3 folds. To improve ALA production, MSGE was diluted to give the COD values of 3,426, 7,644, and 15,288 mg/l to the level present in GSY medium (7,644 mg/l COD) and formulated MSGE was achieved by supplementation with organic acids, glycine, and mineral; that formulated MSGE with nutrients supplementation (formula Q, P, O) (12 folds, 23.5 folds, 46.5 folds produced ALA 24 μM, 22 μM, 15 μM, respectively) could produce ALA higher than formulated MSGE (formula K, J, I) (12 folds, 23.5 folds, 46.5 folds produced ALA 19 μM, 8 μM, 5 μM, respectively). Thus this supplementation could give optimized formulated MSGE. For comparison on the ALA production of formula Q (12 folds diluted with nutrients supplementation; 15,288 mg/l COD) with the another formulated MSGE (with supplementation) (formula O, P, R), formula Q will be selected for production media because it gave the highest ALA concentration and highest dry cell weight in 24 μM and 1.8736 g/l, respectively (Fig. 8(e), 9(e)) that may be its organic matter content was appreciated for bacterium growth and ALA formation.

Formula M, O, P, Q (251.63, 15.22, 21.88 and 23.74 μM, respectively) produced ALA higher than formula G, I, J, K (22.41, 5.33, 8.33 and 18.6 μM, respectively) thus nutrients supplementation induced ALA production and nutrients supplementation did not affected for ALA production in MSGE (waste media) that may be some organic matter in MSGE suppressed nutrients or inhibited ALA synthase in MSGE, then dilution of MSGE could be the way to increase ALA production.

From above, C/N ratio of media was found to be between 9,675 to 54,600 (Fig. 7c) and the selected production medium for this research work was formula Q (12 folds dilution), which gave C/N ratio of 49,316.

4. CONCLUSIONS

1. Monosodium glutamate effluent (MSGE) was selected for cultivation of *R. capsulatus* SS3, and extracellular ALA production (0.20 μM).
2. Supplementation of MSGE (Media Y) with 40 mM succinic acid, 0.5 g/l propionic acid, 10 mM glycine, 15 mM $MgCl_2$ and 15 mM levulinic acid (Media Y) gave higher ALA concentration than formula MSGE (Media X) because glycine and succinic acid are the substrate for ALA production; propionic acid is a cofactor; $MgCl_2$ is a trace element; and LA is an inhibitor of ALA dehydratase.
3. Addition of 15 mM levulinic acid (LA) increased ALA production as LA is an inhibitor of ALA dehydratase.
4. The C/N ratio range for *R. capsulatus* SS3 cultivation was between 9,675 to 54,600 and the C/N ratio for *R. capsulatus* SS3 in formulated MSGE (12 folds dilution) was 49,316.
5. Formulated MSGE (12 folds diluted and nutrients supplementation; formula Q) was the production media since its contains much appropriate organic matter more than the MSGE.

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