Changes in the Microflora and Physiological - Biochemical Characteristics in the Culture of EM

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Abstract
Microflora of EM_1 culture is converted from aerobic and neutral to anaerobic and acidic. Biochemical characteristics of EM_1 changed according to change of microflora. pH, EC and content of organic acid of EM_1 at the end of culture were 3 - 3.5, 0.6 - 0.9 s/m and 1.9 - 2.1 %, respectively. Antioxidation activity of EM_1 increased in proportion to log-phase of effective microorganisms. Antioxidation activity of EM_1 depended more upon antioxidation activity of organic acid than SOD activity. EM_2 and EM_3 had high antioxidation activity and EM_2 had antibiotic action.

Introduction
Changes of EM microflora and physiological-biochemical characteristics of EM culture were investigated and biological activities of EM_2 and EM_3 were tested.

Materials And Methods
Inoculator: EM_1,EM_2, EM_3 from Japan (1996) were used as inoculators for their mass cultivation.
Substrates: Molasses (6 percent) was used for the mass cultivation of EM_1. Fish-fermented juice (0.6 percent), for EM_2 and EM_3.
Small and large-scale cultures of EM_1, EM_2 and EM_3 were inoculated in molasses medium by 3.3 and 5 percent respectively. It was left at 30 ± 2 °C in aerobic condition for the first five days then in anaerobic condition, for about three weeks for mass cultivation of EM_1.
The number of lactic acid bacteria, yeasts, photosynthetic bacteria, N_2 fixing bacteria, and actinomycetes were counted by dilution plate methods in each selective media.
pH, EC and DO of the cultures were measured with TOA pH meter and EC meter.
The content of organic acids was measured by alkaline titration, the content of volatile acids by steam distillation and the content of ester by determination of saponification value of organic acid produced from hydrolysis of ester. The anti-oxidation activity (SOD) was estimated by suppressive degree of spontaneous oxidation of ascorbic acid.

Results and Discussion
Some Physiological-Biochemical Characteristics of Inoculator and Substrate
Some physiological-biochemical characteristics of EM_1, EM_2 and EM_3 used as inoculator are shown in Table 1.

Table 1. Physiological-Biochemical Characteristics of Inoculators, EM_1, EM_2 and EM_3.

Index	EM_1	EM_2	EM_3
PH	3.5	8.7	7.6
EC (s/m)	0.866	0.528	0.277
Refrative index (%)	3.5		
Number of total microbes (cells/ml)	$8\pm2x10^8$		
Content of organic acids (%)*	1.73		
Content of volatile acids (%)**	0.39		

* Latic acid, ** Acetic acid

Some physiological-biochemical characteristics of substrates are shown in Table 2.

Table 2. Physiological-Biochemical Characteristics of Molasses and Fish-Fermented Juice.

Index	Molasses	Fish-fermented Juice
Moisture (%)	9.4	50.6
Content of total saccharides	66.0	
Content of reductive saccharides (%)	8.0	
Content of crude cellulose (%)	4.3	
Content of crude protein (%)		20.6
Content of crude fat (%)	3.2	
Content of ash (%)	8.1	

Changes of Microflora with Incubation Time in EM_1

The microflora and characteristic of EM were changed during the co-cultivation of inoculators (EM_1, EM_2 and EM_3) in the laboratory. (Figure 1)

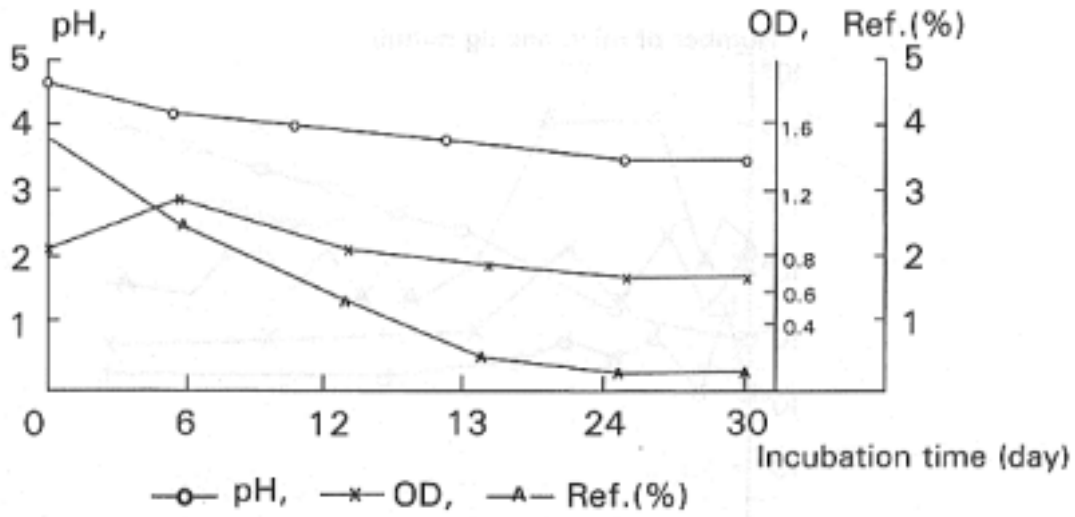
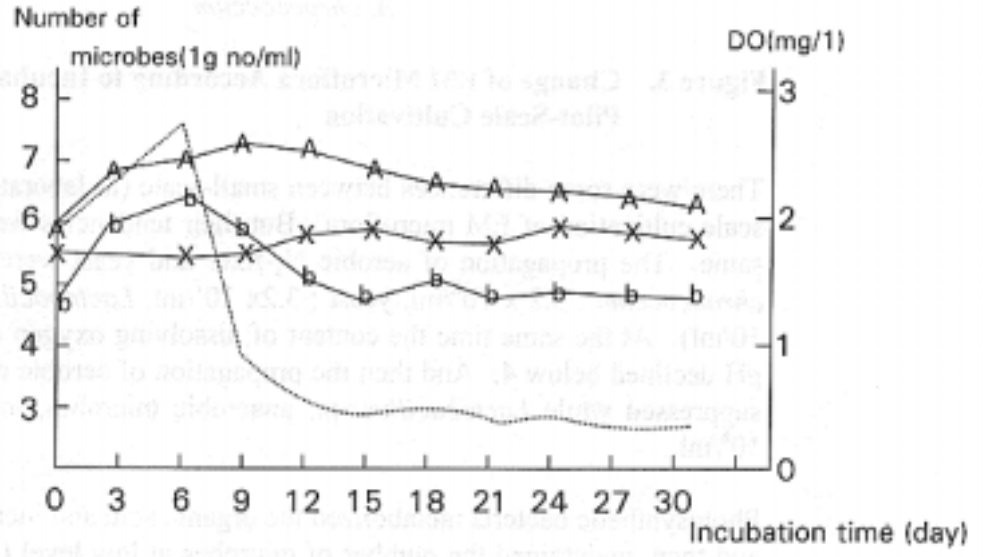


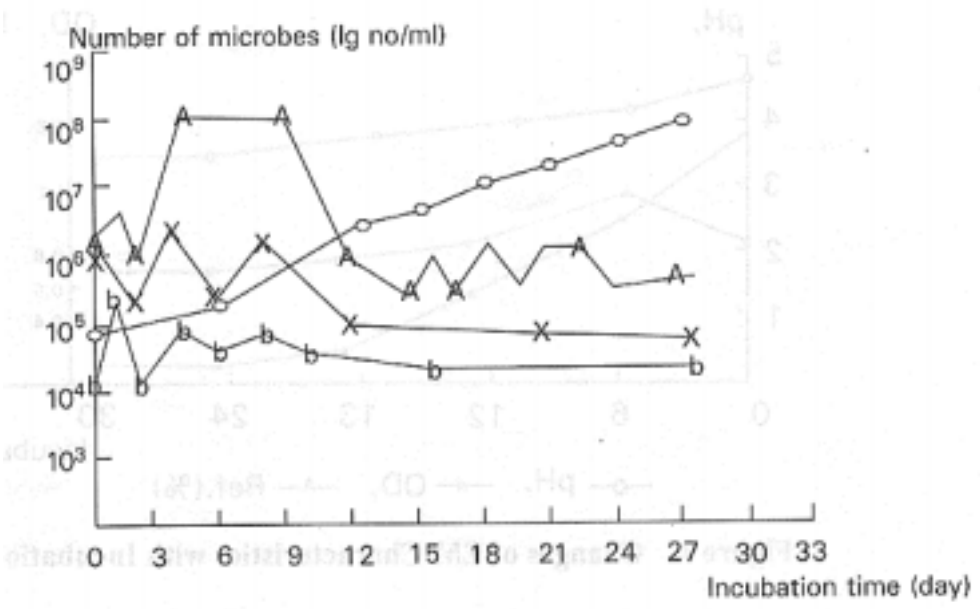
Figure 1. Changes of EM Characteristics with Incubation Time.

pH of EM cultures decreased by 3.0 – 3.5 and EM microflora was changed (Figure 2 and 3).



Lactobacillus sp. Rhodobacter sp. Saccharomyces sp A. chrocooccum

Figure 2. Change of EM Microflora According to Incubation Time at Laboratory.



Lactobacillus sp. Rhodobacter sp. Saccharomyces sp A. chrocooccum

Figure 3. Change of EM Microflora According to Incubation time in Pilot-Scale Cultivation

There were some differences between small-scale (in laboratory) and pilot scale cultivation of EM microflora. But their tendencies were almost the same. The propagation of aerobic N_2-fixer and yeast were observed (*A. chroococcum* : 5.2 x 10^6/ml, yeast : 3.2x 10^7/ml, *Lactobacillus sp.* : 5.1 x 10/ml). At the same time the content of dissolving oxygen decreased and pH declined below 4. And then the propagation of aerobic microbes were suppressed while *Lactobacillus sp.,* anaerobic microbes, increased up to 10^8/ml.

Photosynthetic bacteria metabolized the organic acid and increased slightly and then, maintained the number of microbes at low level (5.4 x 10^5/ml). However, actinomyces were very low (2.1 x 10^3/ml) and didn't observe their propagation.

The antioxidation activity (SOD) rapidly declined after the peak on 6th day of incubation. It increased slightly and maintained at low level.

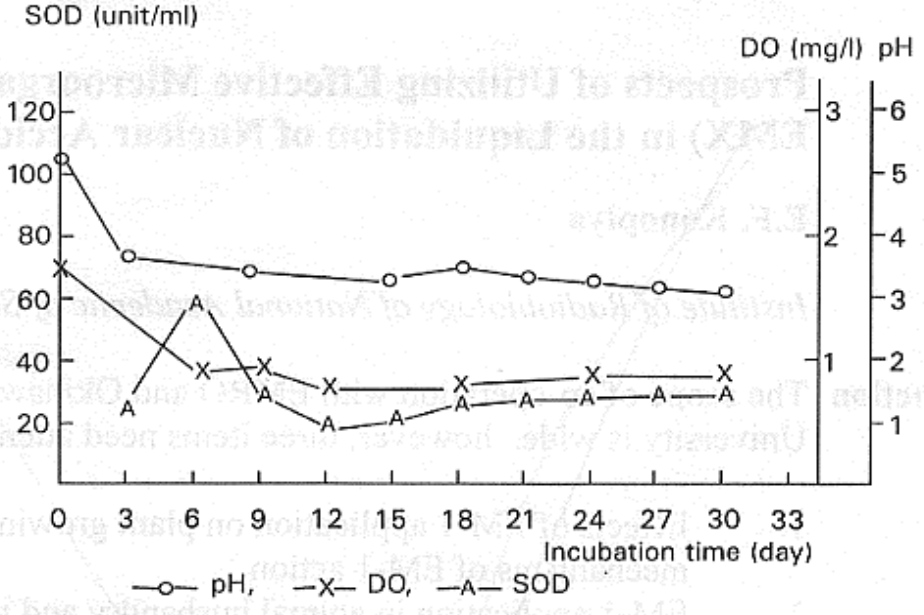


Figure 4. Changes of SOD Activity and pH of EM Culture

The contents of dissolving oxygen and pH decreased, SOD increased slightly (Figure 5).

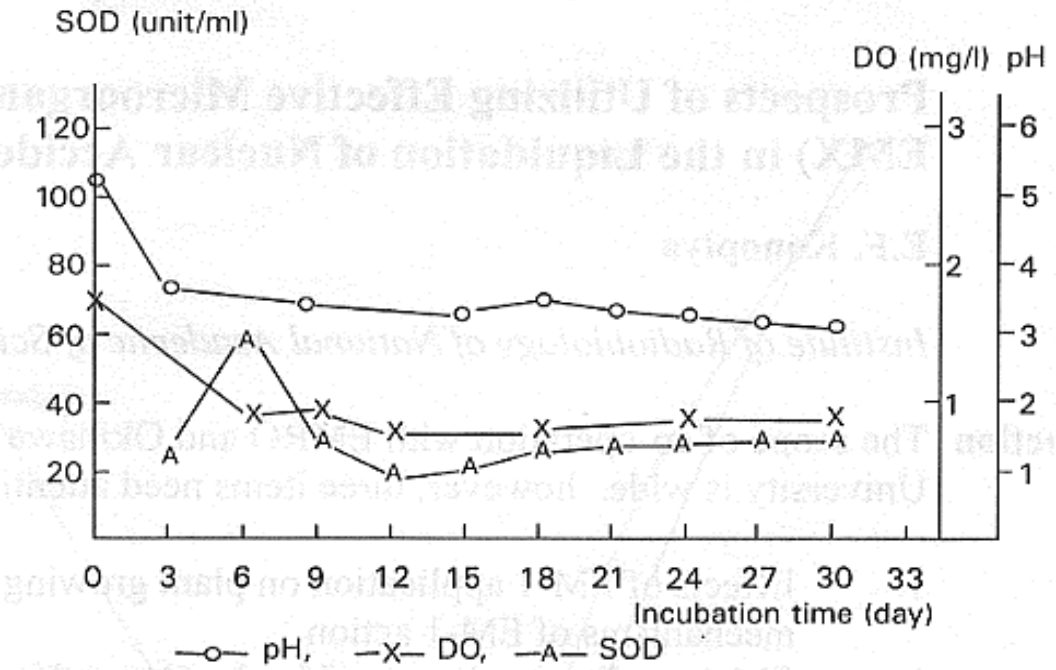


Figure 5. Changes of Total Content of Produced Organic Acid and SOD Activity in EM Culture.

References

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