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Yeast using for Color Removal in Wastewater

Jaruwan Chutrtong, Waradoon Chutrtong

Abstract*: Molasses wastewater is waste from alcohol beverage industry which is huge industry. Its properties are high pollution. If released to environments without treatment, it can cause a lot of environmental problems. So, there are varieties methods for molasses wastewater treatment which highly effective in reducing the COD and BOD levels of the residue to the level that does not pose any problem to the environment. However, molasses wastewater contains difficult to remove melanoidin substance which causes dark colors. So the treated water is still dark. If discharged treated water to natural water sources, the water will be darker. The use of microorganisms in wastewater treatment is a costly method and is relatively safe, even take a long time. This research is to study the optimum conditions for microorganism usage to remove dark color from wastewater by biological methods. Three yeasts, Citeromyces siamensis, Issatchenkia orientalis and Saccharomyces cerevisiae, were selected for this study. Conducted the experiment by adding 1-3 % of glucose, sucrose, lactose, yeast extract, peptone and urea in synthetic molasses wastewater to find the appropriate ratio that will promote molasses wastewater color reduction of those yeast species. Cultured at 30 degrees Celsius for 7 days and measure color intensity with spectrophotometer at wavelength 660 nm. It is found that the addition of 1 percent glucose resulted in the best color loss by all three yeasts compared to the addition of sucrose and lactose. It is also found that the addition of 1 percent peptone gave the best color removal efficiency by Citeromyces siamensis and Issatchenkia orientalis compared to the addition of peptone and urea. While Saccharomyces cerevisiae is effective in reducing the color of molasses wastewater when adding yeast extract 1 percent.

***Index Terms*: Decolorization, Molasses, Wastewater, Yeast.**

I. INTRODUCTION

Molasses is one of the raw materials in alcohol production. Wastewater from the production of alcohol using molasses has many substances which cause pollution to environments and one is its dark brown to black color. The dark color of wastewater resulted from melanoidin which formed by the combination of sugar and amino acids at high temperatures through a browning reaction or a maillard reaction. It can make more dirty and darker to water source. This problem is solved in a variety ways including the use of microorganisms to remove the color but it is difficult because melanoidin is hard-biodegradable substance.

Microorganisms that have been studied include both bacteria and fungi [1]. Miranda et al. [2] studied the color removal of molasses wastewater using *Aspergillus niger*. It was found that 69 % of the residue color was removed and 75 % COD was reduced. Benito et al. [3] studied the color removal of molasses wastewater by using Trametes versicolor. It was found that 82% of the residue color was removed and 77 % COD was reduced. Jiminez et al. [4] studied the method of wastewater treatment by using aerobic and anaerobic system that uses 4 strains of fungus. Sirianuntapiboon et al. [5] [6]selected 205 strains of yeast from Thailand fruit samples and found some strain had the highest color removal capacity of 68.91 % at 30°C. Sirianuntapiboon studied 170 species of acetogenic bacteria and found that species which able to remove maximum color of 76.4 ± 3.2 % at 30°C. Raghukuma et al. [7] [8] studied the melanoidin removal by whitewash fungi, *Flavadon flavus*. By 10 % cell immobilization with polyurethane foam, *Flavadon flavus* was able to remove 60-70 % of color. According to the previous research, it indicates that there are many microorganisms that have the ability to degrade substances which cause wastewater color. *Citeromyces siamensis* and *Issactchenkia orientalis* are yeast which can found in environment of Thailand. *Citeromyces siamensis* is a halotolerant yeast, while *Issactchenkia orientalis* has been studied for the use of fungicide in plant [9]. *Saccharomyces cerevisiae* is used to produce many kinds of beverages and food. These yeasts can grow in the presence of sugar, including molasses. Therefore, it is possible to culture these yeasts to eliminate contaminants in waste water. This study was conducted determining the suitable conditions of medium in testing the efficiency of *Saccharomyces cerevisiae*, another familiar yeast species, for molasses wastewater decolorization compare with *Citeromyces siamensis*, *Issatchenkia orientalis*. This is a more effective treatment of using microorganisms in water pollution removal.

II. PROCEDURE

Study on the optimum conditions for the reduction of molasses wastewater by comparing the appropriate carbon sources and nitrogen sources.

A. Appropriate carbon sources

Prepare yeast starter by Inoculate yeast to MYGP broth which add synthetic molasses wastewater 30 ml. Incubate at 30 ° C for 24 hours. Then measured the absorbance with a spectrophotometer at wavelength 660 nm. The desired value is 0.5. [10]

Add 1.5 ml (5%) of the yeast solution to 30 ml of synthetic molasses wastewater which has 1%, 2% and 3% of sucrose, lactose and glucose. Incubate at room temperature for 1-7 day in 200 rpm shaker [11]. At the end of each period, picked sample. Then analyzed by centrifugation at 5,000 rpm for 20 min. Kept supernatant. Diluted 1 ml. of supernatant with 0.1 M acetate buffer. Measured the absorbance by spectrophotometer at 475 nm.

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***** Correspondence Author
Jaruwan Chutrtong*, Industrail Microbiology, Suan Sunandha Rajabhat University, Bangkok, Thailand,
Waradoon Chutrtong, Chemistry, Srinarinwirot University, Bangkok, Thailand,

B. Appropriate nitrogen sources

Analyze the same as in A. but vary nitrogen sources instead carbon source by adding 1%, 2% and 3% of urea, peptone, and yeast extract. Measured the absorbance at 475 nm. at the end of each period.

III. EXPERIMENTAL RESULT

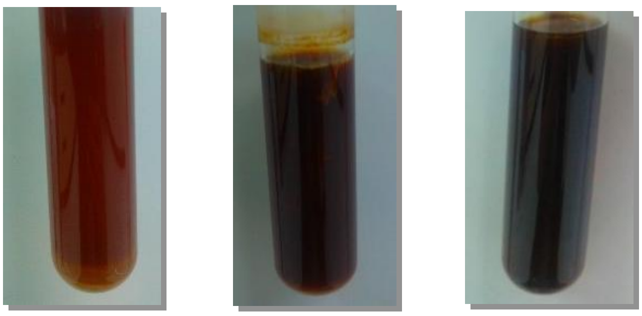
A. Appropriate carbon sources

Table 1, 2. and 3. showed results of carbon sources on the reduction of the wastewater color matter by *Citeromyces siamensis*, *Issatchenkia orientalis* and *Saccharomyces cerevisiae*.

Table 1. Results of carbon sources on wastewater color by *Citeromyces siamensis*

Carbon source	OD at 475 nm of wastewater at room temperature during cultivated period (days)							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1% Gl	4.42	4.22	3.96	3.35	2.96	2.43	2.18	1.93
2% Gl	4.63	4.43	4.25	3.94	3.55	2.52	2.23	2.04
3% Gl	4.48	4.62	4.34	3.96	3.46	2.82	2.52	2.34
1% Su	4.46	4.33	4.06	3.22	2.83	2.34	2.16	2.08
2% Su	4.63	4.53	4.23	3.63	3.15	2.69	2.51	2.35
3% Su	4.84	4.67	4.08	3.77	2.97	2.74	2.66	2.53
1% La	4.45	4.34	4.14	3.63	3.03	2.63	2.41	2.24
2% La	4.64	4.40	4.04	3.73	3.16	2.72	2.58	2.44
3% La	4.79	4.59	4.23	3.62	3.15	2.97	2.62	2.58

Gl = Glucose Su = Sucrose La = Lactose



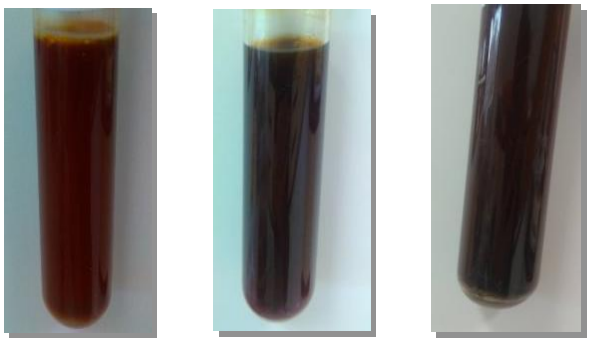
(a) (b) (c)

Fig. 1. The color of wastewater when cultivated with *Citeromyces siamensis* by adding 1% of glucose (a), sucrose (b) and lactose (c).

Table 2. Results of carbon sources on wastewater color by *Issatchenkia rientalis*

Carbon source	OD at 475 nm of wastewater at room temperature during cultivated period (days)							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1% Gl	4.46	4.22	4.01	3.57	2.97	2.15	1.81	1.75
2% Gl	4.65	4.58	4.35	3.92	3.18	2.35	2.14	2.03
3% Gl	4.81	4.66	4.23	3.73	3.48	2.78	2.36	2.36
1% Su	4.51	4.37	4.05	3.67	3.12	2.44	2.16	1.93
2% Su	4.78	4.53	4.31	3.84	3.23	2.97	2.51	2.36
3% Su	4.98	4.77	4.34	4.02	3.77	3.14	2.75	2.57
1% La	4.53	4.35	4.12	3.78	3.24	2.86	2.66	2.22
2% La	4.66	4.43	4.22	3.84	3.57	2.77	2.52	2.38
3% La	4.74	4.56	4.36	3.92	3.57	3.04	2.77	2.56

Gl = Glucose Su = Sucrose La = Lactose



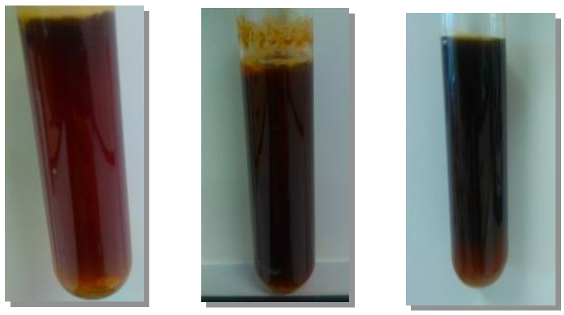
(a) (b) (c)

Fig. 2. The color of wastewater when cultivated with *Issatchenkia orientalis* by adding 1% of glucose (a), sucrose (b) and lactose (c).

Table 3. Results of carbon sources on wastewater color by *Saccharomyces erevisiae*

Carbon source	OD at 475 nm of wastewater at room temperature during cultivated period (days)							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1% Gl	4.44	4.23	3.95	3.52	2.84	2.51	2.36	2.12
2% Gl	4.61	4.48	4.26	3.82	3.41	2.53	2.37	2.29
3% Gl	4.79	4.52	4.35	4.04	3.55	2.93	2.68	2.43
1% Su	4.42	4.23	4.03	3.63	3.13	2.52	2.32	2.12
2% Su	4.56	4.36	4.22	3.46	3.03	2.72	2.55	2.35
3% Su	4.73	4.56	4.34	4.06	3.64	2.72	2.42	2.63
1% La	4.44	4.25	4.04	3.52	3.16	2.78	2.56	2.36
2% La	4.62	4.43	4.16	3.74	3.54	3.02	2.72	2.57
3% La	4.74	4.58	4.37	4.03	3.66	3.13	2.86	2.68

Gl = Glucose Su = Sucrose La = Lactose



(a) (b) (c)

Fig. 3 The color of wastewater when cultivated with *Saccharomyces cerevisiae* by adding 1% of glucose (a), sucrose (b) and lactose (c).

B. Appropriate nitrogen sources

Table 4. 5. and 6. showed results of nitrogen sources on the reduction of the wastewater color matter by *Citeromyces siamensis*, *Issatchenkia orientalis* and *Saccharomyces cerevisiae.*

Table 4. Results of nitrogen sources on wastewater color by *Citeromyces siamensis*

N	OD at 475 nm of wastewater at room temperature during cultivated period (days)

source	0	1	2	3	4	5	6	7
1% Y	4.46	4.56	4.13	3.56	2.94	2.65	2.34	2.14
2% Y	4.55	4.35	4.12	3.64	3.22	2.82	2.51	2.35
3% Y	4.69	4.43	4.03	3.65	2.93	2.53	2.31	2.49
1% P	4.62	4.51	3.92	3.41	2.63	2.36	2.16	2.01
2% P	4.41	4.25	4.02	3.52	2.93	2.77	2.58	2.36
3% P	4.78	4.53	4.04	3.72	3.16	2.95	2.74	2.56
1% U	4.47	4.34	4.07	3.64	3.03	2.71	2.42	2.26
2% U	4.48	4.32	4.14	3.76	324	2.96	2.67	2.43
3% U	4.71	4.37	4.33	3.83	3.14	2.91	2.83	2.64

Y = Yeast extract P = Peptone U = urea

(a) (b) (c)

Fig. 4. The color of wastewater when cultivated with *Citeromyces siamensis* by adding 1% of yeast extract (a), peptone (b) and Urea (c).

Table 5. Results of nitrogen sources on wastewater color by *Issatchenkia orientalis*

N source	OD at 475 nm of wastewater at room temperature during cultivated period (days)							
	0	1	2	3	4	5	6	7
1% Y	4.43	4.26	4.04	3.73	3.44	2.75	2.28	2.14
2% Y	4.67	4.52	4.41	3.95	3.66	2.93	2.58	2.44
3% Y	4.74	4.56	4.32	3.95	3.52	2.45	2.77	2.63
1% P	4.47	4.24	4.07	3.78	3.34	2.36	2.16	1.93
2% P	4.65	4.43	4.24	3.63	3.02	2.66	2.31	2.26
3% P	4.79	4.61	4.43	4.06	3.65	2.95	2.62	2.43
1% U	4.52	4.34	4.11	3.76	3.33	2.54	2.26	2.16
2% U	4.73	4.54	4.37	4.08	3.55	2.82	2.57	2.37
3% U	4.91	4.85	4.64	4.23	3.72	3.03	2.63	2.58

Y = Yeast extract P = Peptone U = urea

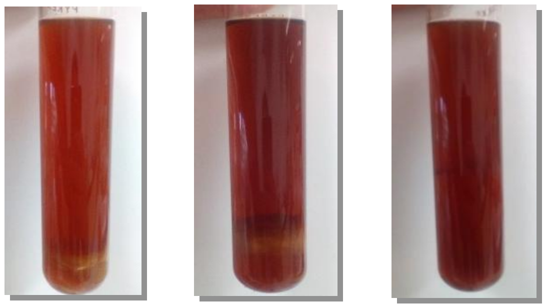
(a) (b) (c)

Fig. 5. Color of wastewater when cultivated with *Issatchenkia orientalis* by adding 1% of yeast extract (a), peptone (b) and urea (c).

Table 6. Results of nitrogen sources on wastewater color by *Saccharomyces cerevisiae*

N source	OD at 475 nm of wastewater at room temperature during cultivated period (days)							
source	0	1	2	3	4	5	6	7
1% Y	4.45	4.23	4.05	4.37	3.57	2.72	2.42	2.22
2% Y	4.44	4.24	4.04	3.66	3.25	2.91	2.63	2.44
3% Y	4.64	4.48	4.15	3.85	3.45	3.05	2.77	2.52
1% P	4.47	4.23	3.93	3.66	3.13	2.96	2.66	2.42
2% P	4.52	4.32	4.12	3.73	3.03	2.76	2.46	2.34
3% P	4.79	4.62	4.34	4.02	3.56	2.96	2.74	2.58
1% U	4.43	4.24	3.93	3.45	3.13	2.84	2.67	2.49
2% U	4.58	4.36	4.06	3.75	3.43	3.02	2.73	2.57
3% U	4.71	4.56	4.34	4.97	3.14	2.83	2.54	2.74

Y = Yeast extract P = Peptone U = urea



(a) (b) (c)

Fig. 6. Color of wastewater when cultivated with *Saccharomyces cerevisiae* by adding 1% of yeast extract (a), peptone (b) and urea (c).

IV. CONCLUSION

Based on experimental results, it was it was found that all 3 types of yeasts could reduce the color of wastewater. The results also shown that adding glucose culminate on the reduction of molasses wastewater color. The ratio that gives the best results was glucose 1%. In the same way, 1% of peptone gave the best effective in reducing the color of molasses wastewater for *Citeromyces siamensis* and *Issatchenkia orientalis* while 1% yeast extract effected in reducing the color of molasses wastewater for *Saccharomyces cerevisiae*. From the results, the addition of little amount of glucose, peptone and yeast extract improves the efficiency of color remove of *Citeromyces siamensis*, *Issatchenkia orientalis* and *Saccharomyces cerevisiae*. The addition of those substances in large quantities does not help to achieve higher efficiency than filling small quantities. Although the color still remains, it decreases the intensity. This finding may be apply for industrial waste water treatment. Since contaminated substances are difficult to decompose, as mentioned above, the treatment for clean water may require other factors to help. Finding other conditions is that should be studied further.

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AUTHORS PROFILE

Jaruwan Chutrtong. Birth place: Bangkok Thailand. Bachelor degree: Bachelor of Science (B.Sc.) in biology (microbiology) from Srinakharinwirot university, Bangkok Thailand in 1985. Master degree: Master of Science (M.Sc.) in microbiology from Kasetsart university, Bangkok Thailand in 1994.

She worked as MEDICAL SCIENTIST in Regional Medical Sciences Center in Nakhon Ratchasima between 1985-1988. In 1988-1994, she worked as MEDICAL SCIENTIST in Department of Medical Sciences, Ministry of Public Health in Bangkok. After that, from 1994 – now, she is LECTURER in industrial microbiology department, faculty of science and technology Suan Sunandha Rajabhat university, Bangkok Thailand. Previous publications:

1. J. Chutrtong, "Comparison of Corncob and Corn Using as Fungal Culture Medium," Procedia-Social and Behavioral Sciences, vol.197, pp. 797-800, 2015.

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Asst. Prof. Jaruwan Chutrtong: memberships – committee of Educational Professional Foundation.

Waradoon Chutrtong Birth place: Bangkok Thailand. Bachelor degree: Bachelor of Science B.Sc.) in chemistry from Ramkhamheng university, Bangkok Thailand in 1984. Master degree: Master f Science (M.Sc.) in chemistry (analytical) from Kasetsart university, Bangkok Thailand in 1995.

He worked as MEDICAL SCIENTIST in Regional Medical Sciences Center in Nakhon Ratchasima between 1986-1989. In 1989-1995, he worked as MEDICAL SCIENTIST in Department of Medical Sciences, Ministry of Public Health in Bangkok. After that, from 1995 – now, he is LECTURER in chemistry department, faculty of science, Srinakharinwirot university , Bangkok Thailand.

Asst. Prof. Waradoon Chutrtong: memberships – committee of Council of science and technology professionals.