INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

A preliminary study on the effect of *Monascus purpureus* inoculation on Melanoidins containing waste

Nandang Suharna

Research Center for Biology, Indonesian Institute of Sciences

Jl Raya Cibinong Km 46 Cibinong Sciences Center, Cibinong, Indonesia.

ABSTRACT

A preliminary study on the effect of inoculation of *Monascus purpureus* NGK on change of chemical composition of waste-containing melanoidins was carried out. Parameters were determined were reducing sugars, total soluble solid (TTS), color intensity (OD 475nm), pH, yellow (OD 420nm) and red pigment (OD 490nm). The result showed that the fungus used reducing sugar. The fungal growth did not affect decolorization and its fungal pigments might be already degraded at high pH level. The result showed that the fungus did not degrade the MCW. It is suggested that more nutritional or chemical addition or adjustment of pH medium was needed to optimize the growth of *Monascus purpureus* so as to degrade and decolorize MCW also to produce its pigments might be achieved.

Key words: inoculation, *Monascus purpureus,* melanoidins and waste.

INTRODUCTION

A large quantities of melanoidins-containing waste (MCW), a by-product of the sugar cane industry are dischargedwhich caused contamination and disruption of the water. This waste has brown color which is derived from melanoidins; Melanoidins are the major component of this waste. As the major organic contaminant, melanodins contribute over 2% of vinasse composition. melanoidins are formed at purification and evaporation steps during sugar processing[1].

Melanoidins is compounds which formed by Maillard reaction, a non-enzymatic reaction which result a complex polymeric compounds which are formed in basic conditions when amino acids and sugars are heated[2].

Successful attempt to eliminate the material which is the main color of waste containing melanoidins also reported[3]. That attempt was a biological system processing by using the 'Activated sludge' which combined with chemical treatment by addition of $FeCl_3$ and Al_2 $(SO)^2$. This treatment managed to eliminate the main colorant and chemical properties, such as COD, BOD_5 and TSS[3]. Many attempts also had been done to deal with this waste by using biological or chemical treatment. Successful biological decolorisation by using fungi such as *Aspergillus*, *Coriolus* and Phanerochaete have been reported[4].

This similar attempt was aimed at determining the influence of inoculating *Monascus purpureus* to waste containing melanoidins. The use of *Monascus* was based on its pigments production for industrial application. There was possibility of the pigments instability during incubation particularly by light or pH changes towards its degradation[5].

The objective of this study was to know the effect of *Monascus* inoculation on chemical changes of melanoidins-containing waste and the fungal pigment production.

MATERIALS AND METHODS

Fungal Strain and Its Cultivation

Monascus purpureus NGK was used in this physiological study was a collection at laboratory microbiology for health at Research Center for Biology, Indonesian Institute of Science. The fungus was cultivated on extract of mung bean sprout (*Phaseolusradiatus* L.) agar 6% (MB 6%). This

fungus was originated from Chinese red rice in Bogor, Indonesia.

Inoculum Preparation

The fungus (*M. purpureus*) was cultivated on Petri plate containing MB 6% medium and incubated for 2 weeks at a temperature of 25°C. Fungal inoculum was agar plug form that was made by using a hole puncher (0.5 mm in diameter) at the edge of the actively growing fungal colony.

Melanoidins-Containing Waste

Melanoidins-containing waste (MCW) was diluted to have three types of waste solutions. First waste solution with water dilution (M) with the level of dilution of 10, 20, 30, 40 and 50% (M10, M20, M30, M40 and M50), the second waste solution diluted with extracts of sprouts (**MB**) with the level of dilution of 10, 20, 30, 40 and 50% (MB10, MB20, MB30, MB40 and MB50), the third diluted with distilled water (M) with the degree of dilution of 10, 20, 30, 40 and 50% (MYP10, MYP20 , MYP30, MYP40 and MYP50) and supplemented with peptone (0.17%) and yeasts extract (0.17%). All treatments were made in 250 ml of Erlenmeyerflask with each containing 150 ml of MCW solution. All cultures agitated by rotary shaker at room temperature and incubated for six months.

Parameter Used

Reducing sugar total soluble solid, color intensity, pH, red and yellow pigments used as parameters for biochemical analysis.

Reducing Sugar Measurement

Dinitrosalicylic acid (DNS) method is a test reagent that allows detection quantitatively. DNS will reacts with a reducing sugar to form 3-amino-5-nitrosalicylic acid which can measured by using spectrophotometer so the present reducing sugar can be determined of its amount. This DNS method used 0.2 ml of sample pipetted into a clean test tube containing 1.8 mL of distilled water and 2 ml of DNS reagent. The test tube was heated in boiling water for 5 minutes which caused the reaction between glucose in the sample with DNS after was cooled to room temperature, the absorbance of the sample was measured at a wavelength of 540 nm by using a UV-Vis spectrophotometer.

Total Suspended Solid

Total Suspended Solid (s) (TSS) is a residue of total solids are retained by a sieve with a maximum particle size of 2µm or larger than the size of the colloidal particles. Total soluble solid dry weight was measured by weighing wastes produced by drying at a temperature of 105^{o} C for 24 hours. Samples were filtered by using filter paper that previously weighed. The filter was dried to have constant weight reached at temperature of 105ºC. The weight increase of the filter represents the total suspended solids (TSS). The value of TSS was calculated the difference between the total dissolved solids and total solids following the below formula:

$$TSS\ (mg / L) = (A-B)\ X\ 1000 / V,$$

Where,A = weight of filter paper + dry residue (mg),
B = weight of filter paper (mg); V = volume of sample (mL)

Color Intensity and pH

The Color Intensity of MCW was measured at OD 475 nm. Measurement of pH was performed by using a pH meter.

Fungal Pigment

Fungal pigments were measured to know the activity of the fungus. As this fungus normally produced red and yellow pigment, measurement was performed at OD 490 nm and OD 420 nm using a spectrophotometer.

RESULT AND DISCUSSION

In Table 1 showed the measurement of reducing sugar atinitial condition (before inoculation) that MB had highest content than M and MY. The two later had similar reducing sugar content. The decrease of reducing sugar content of all MCS solution after six month incubation at room temperature. MCS-B which was the MCS with addition of Bean medium (MB) showed the highest decrease of reducing sugar content.

The decrease of reducing sugar was indicated the growth of the *M. purpureus*of all MCS medium after its six moth incubation period.

The Total suspended solid (TSS) of MCS solution (M, MB, MY) varied but tended to decrease (Table 1).

It was at interest to know correlation between reducing sugars. After calculation using below formula, the correlation coefficient between reducing sugars decrease and TSS was 0.1469

$$Correl(X,Y) = \frac{\sum (x-\bar{x})(y-\bar{y})}{\sqrt{\sum (x-\bar{x})^2 \sum (y-\bar{y})^2}}$$

This calculation result indicated that between reducing sugars decrease and TSS was no correlation.

This calculation might be not accurate as the reducing sugars, such as glucose were good carbon source for mycelial growth. It was assumed that the fungal growth was not good so as to increase TSS was slightly affected.

Table 1
The content of reducing sugar, total suspended solid, and color intensity of melanoidins-containing waste medium.

Medium	Reducing Sugar			TSS			Color Intensity		
	Initial	End	% of increase	Initial	End	% of increase	Initial	End	% of increase
M10	5.4	2.3	57.8	0.8	1.7	52.9	2.0	2.1	3.5
M20	11.3	4.6	59.3	2.1	1.5	-40.0	3.0	4.0	33.7
M30	16.0	8.1	49.4	4.2	5.7	26.3	3.0	7.0	131.7
M40	27.4	10.8	60.6	16.7	5.2	-221.2	3.0	9.7	223.7
M50	35.8	14.5	59.6	16.1	9.5	-69.5	4.0	13.1	227.8
MB10	40.9	7.5	81.8	3.6	4.5	20.0	2.0	5.3	167.0
MB 20	49.3	8.0	83.8	7.9	6.8	-16.2	3.0	7.6	153.7
MB 30	49.0	5.3	89.2	1.2	2.8	57.1	3.0	4.8	60.7
MB 40	52.0	11.0	78.8	5.6	6.5	13.8	3.0	10.5	250.0
MB 50	72.4	12.9	82.2	12.7	7.1	-78.9	3.7	13.5	267.0
MY10	6.0	2.1	65.0	0.3	1.2	75.0	3.0	3.4	12.0
MY20	13.0	5.0	61.5	2.1	2.8	25.0	3.0	4.4	47.0
MY30	19.4	8.0	58.7	6.2	2.8	-121.4	3.0	7.0	133.7
MY40	26.4	12.5	52.8	14.8	4.4	-236.4	3.0	19.8	560.0
MY50	33.0	11.0	66.7	9.1	7.2	-26.4	4.0	12.8	220.0

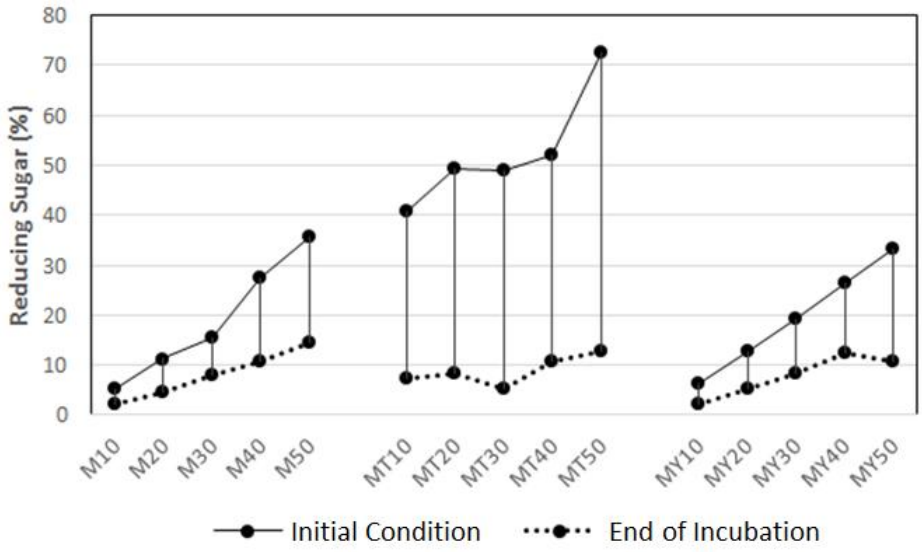
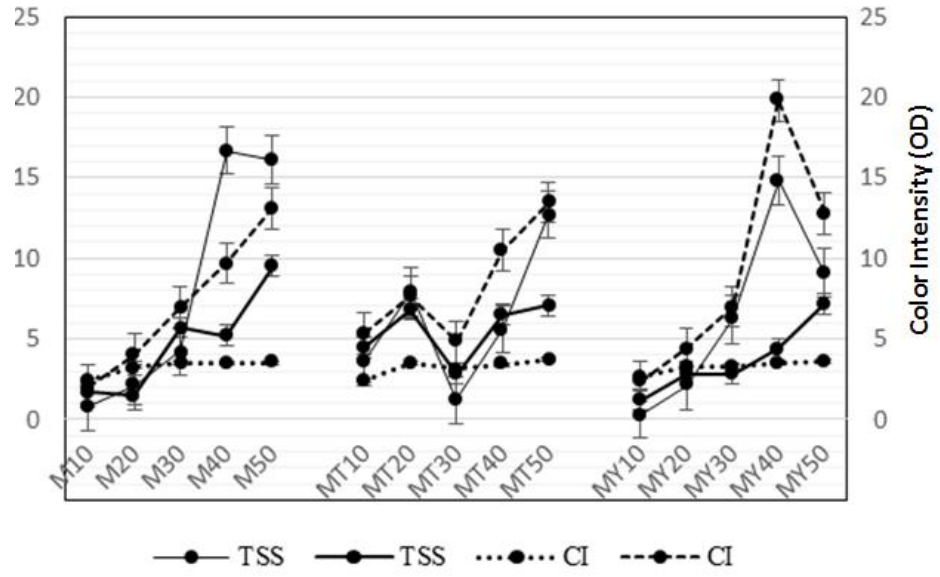


Fig. 1
The effect of Monascus inoculation on changes of reducing sugar of MCS Medium

Table 2
Pigment produced by *Monascus purpureus*.

MCW Medium	Red Pigment		Yellow pigment		pH	
	Initial	End of Incubation	Initial	End of Incubation	Initial	End of Incubation
M10	1.81	1.76	3.97	4.03	6.53	7.7
M20	4.06	3.43	8.44	7.74	6.59	7.5
M30	6.41	5.98	13.51	13.11	6.57	8.0
M40	9.75	8.38	19.6	17.65	6.5	8.1
M50	16.14	11.66	27.76	22.99	6.57	8.3
MT10	1.81	1.76	4.51	4.72	6.17	8.3
MT20	4.06	3.43	8.41	9.28	6.53	8.4
MT30	6.41	5.98	12.33	14.44	6.58	8.3
MT40	9.75	8.38	19.14	18.98	6.2	8.4
MT50	16.14	11.66	25.47	23.36	6.39	8.4
MY10	2.25	2.08	5.44	4.5	7.35	8.3
MY20	4.57	3.75	9.25	8.58	6.73	8.6
MY30	5.84	5.97	12.45	13.14	6.55	8.5
MY40	9.9	9.08	20.08	18.03	6.16	8.4
MY50	11.8	11.43	22.3	22.24	6.47	8.7



Total Suspended Solid (mg/L)
Fig. 2
The Effect of Monascus Inoculation on Changes of Total Suspended Solid and Color Intensity of Melanoidins-Containing Waste Solution

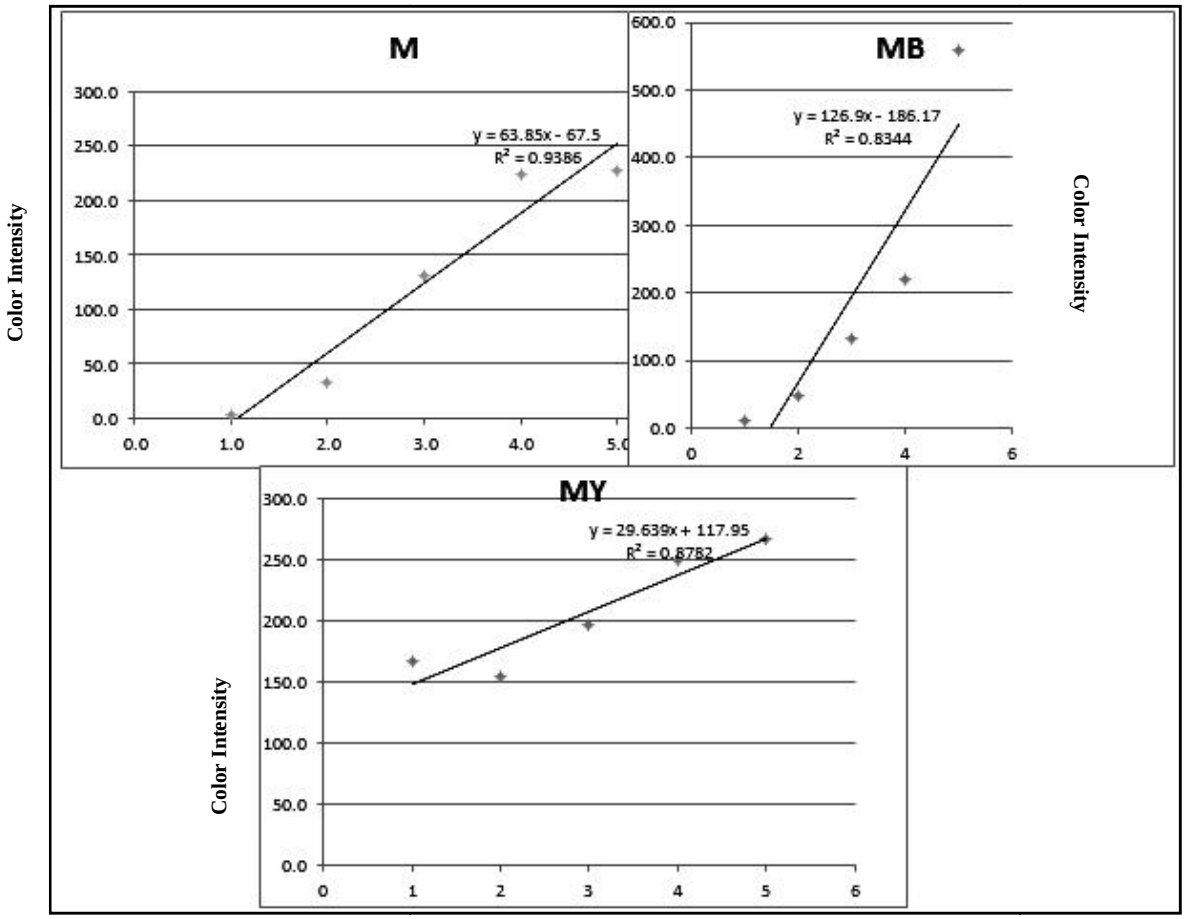


Fig 3
The Effect MCW Medium on Color Intensity

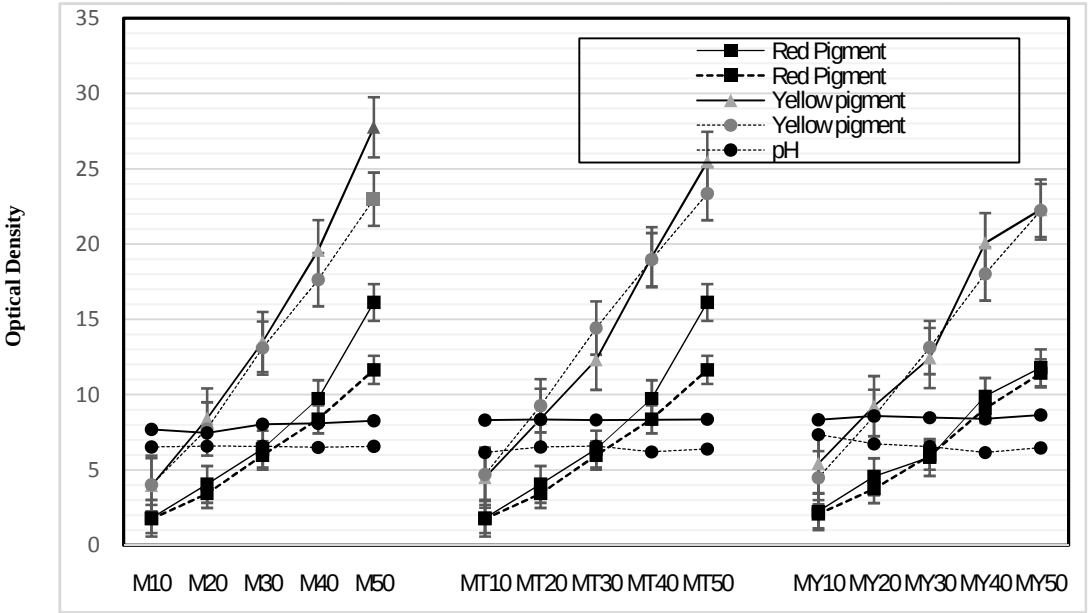


Fig. 4
Red and Yellow Pigment Content of Melanoidins-Containing Waste

The Total suspended solid (TSS) of MCS solution (M, MT, MY) varied but tended to decrease. The color intensity of all MCS solution was increase. While the color intensity increases. The comparison of treatment amongst M, MT and MYP as shown in Figure 1, it seemed that although the content of reducing sugars differ very large but at the end of the incubation period will be obtained by the chemical properties of MCW can be considered similar, as reducing sugar content, TSS, color intensity, pH were not much different from the figure, almost the same.

As the color intensity of all MCS solution was increase, calculation of the correlation with TSS was also calculated and met with coefficient of 0,662338. So, there is positive correlation between TSS and CI.

The color intensity increase represented no decolorization. Although it was also an indication the fungal activity, but It showed that the fungus did not degrade melanoidins but in contrary it added such colorization. These results was in contrast to a report which confirms that a reducing sugar can actually activate the enzymes that degrade melanoidins; Reducing sugars such as glucose and sucrose are suitable for wastewater decolorization dye which has a major melanoidins by fungi Basidiomisetes[7]. The decolorization relates to two types of enzyme activity of intracellular enzymes that are activated by dependency and lack of dependence on the presence of sugars and enzymes which are mainly induced by melanoidins[7].

The results of measurements did show the production of red and yellow pigments by *M. purpureus* of all MCS medium (Table 2 and Fig 3). Generally, glucose is considered as the best carbon source for formation of pigment [5,6]. Therefore, the possibility of no pigments production was caused by degradation at high pH level or by light. *Monascus* pigments are also known unstable towards light which caused rapid degradation of these metabolites[5].

Biological treatment by using fungi including *Aspergillusniger* for decolorization was reported[8]. Decolorization of anaerobically biodigested distillery effluent was mainly composed by melanoidins reported effective by *Aspergillus niger* after pretreatment with alum. This treatment considered a feasible alternative[8].

As microbial enzymes including laccase are able to degrade persistent molecules in contrary to the conventional biological process, their immobilization to overcome the loss of enzyme activity during catalysis of the process, is often a successful strategy[9]. Decolorization of melanoidins by covalently immobilized laccase by 47 % decolorization in 6 h at pH 4.5 and 28 °C[10]. Moreover, 90 % decolorization was achieved after a comprehensive treatment scheme integrating enzymatic, microbial and membrane filtration steps[10]. Similar work by using laccase was reported. After laccase was covalently immobilized on alumina or controlled pore glass-uncoated particles, degradation of simulated molasses wastewaters was effectively reached after 48 h by 74% and 71%, respectively. Degradation of baker's yeast effluents was also effectively reached by immobilized laccase on glass by 68% within 24 h at pH 4.5 and 28 °C for a melanoidin solution 1% v/v[11].

Meanwhile, treatment MCW by coagulants itself was investigated[11]. Decolorization by 92.7% reached after treated by ferric chloride which indicated a preferential removal of high-molecular-weight melanoidins over low weight melanoidins[11]. However, improvement of biodegradability of the treated effluent for it to be reused as dilution water for anaerobic digestion was needed [11].

CONCLUSION

Inoculation of *Monascus purpureus* NGK was not effective on the removal of the primary color materials, melanoidins-containing waste (MCW). It is suggested that more nutritional or chemical addition including treatment pH medium was needed to optimize the growth of *Monascus purpureus* so as to degraded and decolorize MCW also to produce its pigments might be achieved.

ACKNOWLEDGEMENT

Author thanks Rita Dwi Rahayu sincerely for her invaluable assistance during technical analysis.

REFERENCES

1. Arimi MM, Zhang Y, Gotz G, Kiriamiti K, Geisen SU. Antimicrobial colorants in molasses distillery wastewater and their removal technologies. Int. Biodeterior. Biode grad. 2014;87: 34–43.
2. Coca M, Garcı́ MT, González G, Peña M, Garcı́ JA. Study of coloured components formed in sugar beet processing. Food chemistry, 2004;86(3):421-33.
3. Sirianuntapiboon S, Chairittanawan K, Ohmomo S. Removal of colored substances from molasses waste-water by biological treatment systems combined with chemical treatment. *Jarq*,1998;32(3): 211-216.
4. Chandra R, Naresh R, Rai V. Melanoidins as major colourant in sugarcane molasses based distillery effluent and its degradation. *Bioresour. Technol*, 2008; 99(11)**:** 4648–4660.
5. Broder CU, Koehler PE.1980. Pigments production by *Monascus purpureus* with regard

to quality and quantity. J Food Sci, 1980; 45(3):567–569.
6. Arunachalam C, Narmadhapriya D. Monascus fermented rice and its beneficial aspects: a new review. Asian J Pharm Clin Res, 2011; 4(1): 29–31.
7. Ohmomo S, Aoshima I, Tozawa Y, Sakurada N. Purification and Some Properties of Melanoidin Decolorizing Enzymes, P-III and P-IV, from Mycelia of Coriolus versicolor Ps4a. Agric Biol Chem. 1985; 49(7): 2047–53.
8. Shukla SK, Tripathi A, Mishra PK, Nagar GB. Fungal decolorization of anaerobically biodigested distillery effluent (abde) following coagulant. Int J Sci Environ Technol. 2014; 3(2):723–34.
9. R.P. Georgiou, E.P. Tsiakiri, N.K. Lazaridis, A.A. Pantazaki. Decolorization of melanoidins from simulated and industrial molasses effluents by immobilized laccase. Journal of Environmental Chemical Engineering. 2016. 4(1): 1322–1331
10. Singh N, Basu S, Vankelecom IFJ, Balakrishnan M. Covalently Immobilized Laccase for Decolorization of Glucose-Glycine Maillard Products as Colourant of Distillery Wastewater. Appl. Biochem. Biotechnol, 2015; 177(1): 76-89
11. Arimi MM, Zhang Y, Götz G, Geißen SU. Treatment of melanoidin wastewater by anaerobic digestion and coagulation. Environ Technol, 2015; 36(19): 2410–8.