In silico Interaction Studies of Melanoidin Pigments with ligands reveal preferential binding and their plausible roles in bioaccumulation and biomagnification

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ABSTRACT

The presence of melanoidin polymers in the distillery effluent are considered as the main reason for its pitch dark brown colour and its recalcitrant nature. Such distillery effluents when disposed into water bodies lead to rapid dip in the photosynthetic activities and dissolved oxygen contents, gravely affecting the aquatic life. Melanoidin is xenobiotic in nature and the consumption of the dairy products, meat etc., from cattle/livestock sources, which inevitably depend on such contaminated water bodies, affects the health of not only humans, but also many veterinary species. The pumping of industrial effluents into those water sources, eventually results in biomagnification of the heavy metals like Hg, Cd, Pb, Cr, and ligands like acrylamide, arsenic etc. The accumulation of these metals and ligands in such water bodies would trigger polymerization of melanoidin and lead to its buildup in the human system through various food chains. The higher order aggregates of melanoidins are considered as one of the causative agents of several skin allergies, lung ailments, and stomach disorders etc., including colon cancer in humans.

Thus, recognizing the fact that heavy metals and certain ligands amplify the concentrations of melanoidin and activate the conversion of lower molecular weight forms into more hazardous higher order aggregates, interactions studies were carried out to investigate the modes of ligand binding to melanodins. The results delineate the nature of molecular interactions, and highlight their importance inbioaccumulation and biomagnifications, towards designing cost-effective strategies that could be innovatively developed, to solve this perennial environmental risk.

Keywords: *Melanoidin, industrial effluents, bioaccumulation, ligand interactions.*

I. INTRODUCTION

Distilleries are regarded as one among the highly polluting industries worldwide (Kim and Ihm, 2010); they are also rated as one among the 17 most polluting industries listed by the Central Pollution Control Board (CPCB), Govt. of India (CPCB 2009). It is thriving because of the huge demand for alcohol from cane molasses, which is more than 13 million m^3 per annum on a global scale. This sector as a whole generates more than 193 million m^3 per annum of waste water (Khairnar et al., 2013). India maintains about 579 sugar mills and 295 distilleries (Murthy and Chaudhari, 2009) that use sugarcane molasses as a preferred raw material for their annual production of about 2.7 billion litres of industrial alcohol, thereby creating 40.72 billion litres of waste water i.e. spent wash (Saha et al., 2005; Pant and Adholeya, 2006; Belkacemi et al., 2000; Dahiya et al., 2001; Sangve and Pandit, 2004). About 8-10 kgs of molasses are used for producing 1 litre of alcohol, and with this about 10-15 litres of spent wash is produced (Joshi et al., 1996; Rajor et al., 2003). This aqueous distillery spent wash is a dark brown organic effluent which is approximately 12-15 times by volume of the product (Deshpande et al., 2011). Distillery spent wash has extremely high biological oxygen demand (BOD) ranging between 35,000 to 40,000 mgl^{-1} and very high chemical oxygen demand (COD) in the range of 90,000-1,10,000 mgl^{-1} (Santal et al.,2003). This high BOD/COD ratio (0.38-0.36) and high amount of solid wastes (about 82,480 mgl^{-1}) associated with its pungent bad odor makes the spent wash harmful for the ecosystem (Santal et al., 2003).

The presence of melanoidins (which are heterogenous brown polymer formed by Maillard reactions) render these spent wash recalcitrant to any viable remediation procedures, due to their complex structure, xenobiotic nature , as well as both carcinogenic and mutagenic issues (Chandra et al., 2008) behaviors. Higher order polymers of melanoidins make the distillery spent wash very dark brown in colour, that cause eutrophication in natural water bodies, rapid decrease in photosynthetic activity and depletion of dissolved oxygen concentrations (Kumar et al., 1997). Upon discharge of such untreated effluent into water sources it makes the water body unfit for aquatic life (FitzGibbonet al.1998). The environment is also imbalanced because, such contaminated water reduces soil alkalinity and inhibits seed germination (Santal et al., 2003). Stagnation of such distillery effluents on land also results in obnoxious conditions in the region, affecting the porous soil and ground water quality (Jain et al., 2005). Subsequently, the human populations who inevitable depend upon such water sources, begin to feel the hazards and experience symptoms such as severe headache, vomiting sensation, drowsiness, irritation of eyes, skin allergies, dysphagia (difficulty in swallowing the food), sleep disorders, stomach pain, etc. The usage of such untreated water for rearing livestock also leads to morality, low milk yield and depleting health conditions (Chaudhary et al., 2011). The consumption of the dairy products and meat from such cattle/livestock in turn affects the biomagnifications of the hazardous metals like Pb, Hg, Cd, Crin the digestive system of humans and their accumulation to toxic levels (Chaudhary et al., 2011). In addition, the disposal of distillery effluents in the Ganga-Meghan-Brahmaputra plains, which is already contaminated with acrylamide, mercury and arsenic from other industrial effluents, has amplified the health concerns (Chaurasia et al., 2012; Sinha et al., 2007; Buranasilp et al., 2011; Wang et al., 2001). As per EPA 2010 and IRIS 2012 reports, about 2 micrograms of acrylamide per kilogram body weight per day is considered to be toxic, resulting in neurodegenerative disorders (refer Table 2).

The defiance of melanoidins to theirdegradation is apparent from the fact that these compounds escape various stages of wastewater treatment operations, and enter into the environment exhibiting its stealth and harmful effects on planet earth (Pandey et al., 2003). Hence, immediate measures are need of the hour to, not only contain and reduce, but also strategize effective remediation measures to deal with this environmental menace. In order to plan the effective modes of treatment, prior analysis of the biochemistry of melanoidin degradation or its interaction with ligand systems needs to be deeply appreciated. It is known that, in the presence of ligands like acrylamide, arsenic, lead and mercury etc., lower molecular weight forms of melanoidin polymerize into higher order aggregates and cause environmental hazards (Chaurasia et al.,2012; sinha et al.,2007; Buranasilp et al., 2011; Wang et al.,2001).

Thus, with the prior knowledge of the composition of effluents and the composition of toxic compounds in river Ganges basin (as detailed in Table 1 and Table 2), in silico approaches were employed to study the biochemistry of melanoidin-ligand interactions, and their effects on polymerization, bioaccumulation and biomagnifications, so as to design and develop plausible bioremediation processes.

II. MATERIALS AND METHODS

The protocol followed in the study is illustrated as a flow chart in Fig. 1. Ligands known to have defined affinities with the two melanoidin variants were selected based on the percentage of chemical entities present in the distillery effluent and river ganges basin (refer Table 1 and Table 2). As detailed in Table 3, the RMSD between the two variants were 0.412552 Angstroms for all 56 atoms, indicating that the conformations of the two variants are similar.

To determine the modes of ligand interactions, , docking studies were carried out using the Discovery Studio software Version 3.5 (AccelrysSoftwareInc., USA) with the melanoidin variants. The details of interactions are provided in Table 4. The interactions were analysed around 5Å distances from the 14 ligands

III. RESULTS AND DISCUSSION

It is known that melanoidin along with the metals and various ligands are critical for initiation of cancer and mutagenic events. Among the 14 ligands which were docked with the two melanoidin variants, it came to limelight that the polymerization of melanoidin variants appear to get hastened in the presence of substances like arsenic, lead, mercury and acrylamide, as they exhibit higher affinity of binding (as tabulated in Table 4). The interactions of ligands with melanoidin second variant highlight that,in addition to lead and mercury, manganese also appears to bind with better affinities. Thus, quick removal of these ligands from the effluent would itself offer plausible solutions towards preventing bioaccumulation/biomagnifications of carcinogenic substances in the ecosystem. With these clues from the computational exercises, the course of future work is to develop cost-effective bioremediation processes as conceivable technological solutions for the fast removal of these ligands from effluents and enable the spent wash become safe and eco-friendly.

CONFLICT OF INTERESTS

The authors declare that this paper has no conflict of interests with Accelrys Software Inc., USA, version 3.5 software, which is a licensed version.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the generous support and facilities extended by Sir M. Visvesvarya Institute of Technology and Sri Krishnadevaraya Educational Trust, Bangalore, towards this project.

14 ligands known to have affinity in binding with the two melanoidin variants were selected

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3D structures of these 14 ligands were retrieved from the NCBI-PubChem

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3D structures for the 2 melanoidin variants were retrieved based on the empirical formula **C17-18 H26-27O10N C17H26010NPS (First Variant) and C18H27010N (Second Variant)**

⇓

Docking of 14 ligands with melanoidin variants

⇓

Interaction patterns were analyzed to derive the determinants of binding

Fig 1: **Work Flow Chart**

TableI:Composition of Distillery Effluent

Chemical Parameters	**mgl-1**
pH	7.23
Electrical Conductivity(μs)	28700
Total Solids	35340
Total Dissolved Solids	27240
Total Suspended Solids	9980
Settleable Solids	9860
COD	30520
BOD	15300
Carbonate	Negligible
Bicarbonate	12200
Total Phosphorus	28.36
Total Potassium	6500
Calcium	920
Magnesium	753.25
Sulphate	5100
Sodium	420
Chlorides	5626
Iron	6.3
Manganese	1429
Zinc	1.09
Copper	0.265
Cadmium	0.036
Lead	0.19
Chromium	0.067
Nickel	0.145
Ammonical Nitrogen	636.25
Total Phosphorus	29.28
Total Potassium	7300
Sulphur	75.6

Toxic Compounds	**Basis For Selection**	**Toxic Level**

Arsenic	Its presence in the river basin due to the contamination of industrial effluents apart from the distillery effluent	Above 0.05 mgl-1
Mercury	Its presence in traces due to the immersion of the mercury paint coated idols and due to discharge of wastewater from mining activities along the banks of river	0.00023 mgl-1
Acrylamide	Its presence in natural water and soil systems as it is widely used as cement binder and solidification agent	Above 0.002 milligrams per kilogram of body weight per day

Table II: **The toxic compounds in the contaminated water bodies due to discharge from other industrial effluents**

Table III: **Structure and Properties of Melanoidin Ligands (Variants)**

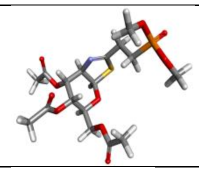
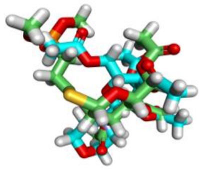
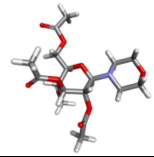
Given Name	**3d Structure**	**Source(Common IUPAC Name)**	**Pub Chem Id**	**Molecular Formula And Molecular Weight(g/mol)**	**Overlay Similarity C17H26010NPS- Green (1st Variant) VS C18H27010 N - Blue (2nd Variant)**
Melanodin first variant		[(5R,6S,7R)-6,7-diacetyloxy-2-(2-dimethoxyphosphorylethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d][1,3]thiazol-5-yl]methyl acetate	59153295	$C_{17}H_{26}NO_{10}PS$ 467.427	**RMSD - 0.412552 Angstroms** 
Melanoidin second variant		[(2R,3R,4S,5R,6R)-3,4,5-triacetyloxy-6-morpholin-4-yloxan-2-yl]methyl acetate	2825736	$C_{18}H_{27}NO_{10}$ 417.407	

Table IV: **Residues of ligands interacting with melanodin within5Å and their strength of interactions**

(Interaction strength with range: up to 3 - less strong; From 3 to 6 – strong; Above 6 – very strong)

Ligands	**C17H26010NPS First Variant**			**C18H27010N Second Variant**		
	No of interactions	**Strength of interactions**	**Details of interactions**	**No of interactions**	**Strength of interactions**	**Details of interactions**
Acrylamide	8	Very strong	3.159 4.352 4.645 4.797 4.578 4.136 4.275 3.891	6	Strong	3.146 4.168 4.574 4.314 3.717 4.656
Arsenic	8	Very strong	4.080 4.018 4.481 3.330 4.721 4.914 4.914 4.993	3	Less strong	4.623 3.397 4.117
Cadmium	4	Strong	2.349 3.786 3.482 4.442	6	Strong	2.245 4.260 4.336 2.459 4.153 4.347
Chromium	4	Strong	2.349 3.786 3.482 4.442	6	Strong	2.245 4.260 4.336 2.459 4.153 4.347
Copper	4	Strong	2.507 4.603 4.435 4.929	6	Strong	2.424 4.289 4.440 2.644 4.374 4.433
Iron	4	Strong	2.501 4.430 4.600 4.930	6	Strong	2.416 4.287 4.437 2.640 4.365 4.433
Lead	8	Very strong	2.493 2.261 2.146 3.319 4.035 4.162 4.417 4.884	7	Very strong	2.133 2.181 3.719 3.673 4.667 4.298 3.935
Manganese	2	Less strong	2.633 4.940	5	strong	4.244 4.788 2.740 3.431 4.750

Mercury	8	Very strong	2.493 2.261 2.146 3.319 4.035 4.417 4.162 4.884	7	Very strong	2.133 2.181 3.719 3.673 4.667 4.298 3.935
Nickel	4	strong	4.225 4.488 4.913 2.320	6	strong	2.299 4.290 4.356 2.458 4.206 4.324
Zinc	4	strong	4.310 4.543 2.401 4.930	6	strong	2.359 4.304 4.385 2.503 4.279 4.337
Calcium	4	strong	2.774 4.715 4.738 4.910	5	strong	4.792 2.680 3.448 4.678 4.225
Magnesium	4	strong	2.526 4.457 4.614 4.930	6	strong	4.451 2.436 2.672 4.391 4.453 4.286
Sodium	3	Less strong	3.125 4.847 3.761	5	strong	3.613 2.587 4.444 2.658 4.083

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