ABSTRACT

Trace elements and vitamins are required for normal growth, development, and proper functioning of the human body. When these nutrients were deficient in the body, it leads to the occurrence of micronutrient malnutrition or hidden hunger which is a formidable issue even in the growing countries. Fortification of food with these vitamins and elements has emerged as a cost-effective technology to combat micronutrient malnutrition. However, the present fortification technologies rely on the addition of inorganic metal salts to food to address trace metal malnutrition. The metal when present in food reacts with the food components and causes nutritional and organoleptic deterioration of the fortified food products. Metal when bound to organic ligands like microorganisms is unavailable for reactions while being bioavailable. Hence microorganisms enriched with metal can be used as a fortificant to overcome nutritional deterioration. Among the trace elements, iron is an essential and vital element for the human body to perform various physiological functions. Besides, deficiency of iron is most prevalent among other trace metal deficiencies. Hence, the present study primarily focused on the characterization of the metal binding potential of food-grade microorganisms for iron to address iron deficiency.

Food grade microorganisms namely, *Lactobacillus fermentum* and *Bacillus subtilis* were used for biosorption of iron, and yeast *Saccharomyces cerevisiae* was used for uptake of iron ions. Batch biosorption and uptake experiments were performed and resulted residual iron was analyzed by Inductive Coupled Plasma Optical Emission Spectroscopy (ICP-OES). From the result the biosorption capacity of *Lb. fermentum* and *B. subtilis* was found to be 7.25 and 7.04 mg per gram of the biosorbent at optimized experimental conditions of 1 g/L of biomass, pH 4.5, 24 h,

and 100 mg/L of ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) respectively. The uptake capacity of *S. cerevisiae* by cultivating with 5 mg of Fe (II) was found to be 3 mg per gram of biomass. Characterization of biomass after biosorption by Scanning Electron Microscope (SEM) equipped with EDX displays the morphological changes and elemental composition. It showed that the iron was bound as bulky particles on surface of the biomass and displayed a Fe peak in the EDX spectrum. Fourier Transform Infrared spectroscopy (FTIR) analysis determined that the functional groups carboxyl, hydroxyl, and amino groups are majorly involved in iron biosorption. Point zero charge (pH_{pzc}) was found to be 3.0 and it is 2.0 for *Lb. fermentum* and *B. subtilis*, respectively. X-Ray Diffraction (XRD) analysis determines the crystallinity of the biomass for biosorption. Modeling of experimental data of *Lb. fermentum* showed the best fit with Freundlich isotherm, while for *B. subtilis* Langmuir isotherm was the best fit. Kinetic data confirm that the pseudo second-order model was the best fit for *Lb. fermentum* and pseudo first-order for *B. subtilis*, respectively.

Exopolysaccharides (EPS) are investigated to possess various human beneficial health effects and their usage in different industries has increased in recent times. Hence, in the present study, the biosorption potential of exopolysaccharide produced by lactic acid bacteria (LAB) was determined for biosorption of iron ions. LAB which can produce EPS were isolated on skim milk lactose medium (SKM). EPS from the strain CC30 was isolated, precipitated, and characterized. The obtained EPS showed the biosorption efficiency of 72.45% at 2 mg/L Fe (II) concentration.

The bioaccessibility and bioavailability of iron from biomass were investigated by simulated gastrointestinal (*in vitro*) digestion and animal studies (*in vivo*) respectively. Both control and iron bound/uptake biomass pellets were subjected to

simulated gastrointestinal digestion with pepsin, pancreatin, and bile salts and the obtained digests were studied by ICP-OES. The result shows that 60.5%, 60.2%, and 65.7% of iron was released after pepsin digestion and 96.3%, 96.1%, and 94.1% of iron was released after pepsin – pancreatin – bile salt digestion from iron bound biomass of *Lb. fermentum* and *B. subtilis* and *S. cerevisiae* respectively. No iron was observed from the control biomass of all the microorganisms. The efficacy of the iron bound biomass in treating iron deficiency anemia and its ability in maintaining the hemoglobin concentration throughout the study was evaluated by a 12-week iron depletion repletion that was performed in 6 groups of 6, male, Wistar rats. Iron deficiency was attained in rats by feeding iron-deficient diet for 8 weeks. After induction of iron deficiency, the rats were given the feed fortified with 35 mg/kg of iron bound *Lb. fermentum* biomass (D1), *Lb. fermentum* biomass without iron (D2), and ferrous sulfate heptahydrate (D3) for 28 days. No significant difference was noticed in the body weight, food intake, hemoglobin concentration, and Hb-Fe pool of the rats in iron bound biomass fortified treatment (T1) and $FeSO_4$ fortified treatment (T3) groups. Also, the concentration of hemoglobin was maintained at normal levels when provided with iron bound biomass throughout the study (FT) as that of the control group fortified with ferric citrate (CG). There was a decrease in hemoglobin concentration in metal-free biomass (T2) and iron-deficient diet (ID) fed groups. The diet fortified with iron bound biomass showed a high relative biological value (RBV) of 118% (T1) and 108.45% (FT) with respect to their control groups (ST and CG). Histology studies of the liver and spleen showed no occurrence of inflammation in any of the treatment groups.

Fortification was done by adding various amounts of free biomass, biomass bound with iron and inorganic iron in the form of ferrous sulfate heptahydrate to milk

(3% and 4.5% fat) and chocolate. Milk was fortified with *Lb. fermentum* and chocolate were fortified with all the three biosorbents. After incubation, the fortified food products were monitored for appearance, microbiological, and oxidative changes (chemical analysis). It was observed that the food fortified with inorganic iron displayed significantly higher TBA values in comparison with the other (iron bound biomass fortified or biomass or control) treatments of curd/dahi/chocolate samples. There was no difference in LAB counts of curd/dahi, but upon storage, in all the treatments there was a slight decrease in counts. The black discoloration was noticed in the inorganic iron fortified chocolate treatment which was absent in other (iron bound biomass fortified or biomass or control) treatments. The intensity of darkening increased with the increase of the storage period. Gastrointestinal (*in vitro*) digestion of fortified chocolate showed that 60%, 61.2%, and 65.9% of iron was bioaccessible after gastric digestion with pepsin and 96%, 95.8%, and 94.5% of iron after gastrointestinal digestion with pancreatin – bile salts from iron bound biomass fortified chocolates treatments of *Lb. fermentum*, *B. subtilis*, and *S. cerevisiae* respectively.

Hence from the study, it was suggested that biomass bound with metal can serve as an efficient fortificant for addressing trace metal malnutrition (Hidden hunger) without any oxidative and nutritional deterioration and with high bioavailability.

Keywords: Trace elements, Iron, Biosorption capacity, Uptake capacity, Biosorption, Characterization, Isotherms, Kinetics, Bioavailability, Fortification, Hemoglobin regeneration efficiency, Relative biological value, Chocolate, Curd, Thiobarbituric acid.