DE GRUYTER Sci Eng Compos Mater 2015; aop

Ashok Kumar Sonia and Keereyadath Priya Dasan*

Feasibility studies of cellulose microfiber (CMF) reinforced poly(ethylene-co-vinyl acetate) (EVA) composites for food packaging applications

Abstract: Recently, there has been increased interest in the extraction of cellulose microfiber (CMF)/nanofibers from plant sources and their utilization as fillers in polymers. These natural fibers are characterized by good mechanical, thermal, and biodegradability properties. The present work was aimed at studying the feasibility of these fiber-reinforced polymer in food packaging. Poly(ethylene-co-vinyl acetate) (EVA) is a common food packaging material used in refrigerated items. The CMF/EVA composite films were exposed to milk, curd, and orange juice at fixed time intervals at three different temperatures and the microbial growth profile in the food material was evaluated. The incorporation of CMF in EVA was found to have no impact on the microbial contamination or proliferation of microbial growth in the food materials.

Keywords: cellulose microfibers; EVA; microbial growth; milk; orange juice.

DOI 10.1515/secm-2014-0252
Received August 4, 2014; accepted November 2, 2014

1 Introduction

Polymer materials are widely used in food packaging and have became an indispensable element in the food manufacturing process. Packaging makes food more convenient and gives the food greater safety assurance from microorganisms and, biological, and chemical changes. However, the rampant use of polymers in food packaging has led to increased disposal problems and serious environment issues. Natural fiber-reinforced composites are an alternative to this issue. The development of cellulose-reinforced polymer material as a replacement for conventional plastic provides many advantages – utilization of an abundant supply of celluloses, reduction of the existing dependence on non-renewable resources, and reduction of plastic waste and associated harmful substances from the process of plastic incinerations.

Natural fibers are made up of repeated crystalline structures resulting from the aggregation of cellulose chains. Each fiber is essentially a composite in which rigid cellulose microfibrils are embedded in a soft matrix mainly composed of lignin and hemicelluloses. The degradation of non-cellulose content gives rise to a new form of fibers, cellulose microfibers (CMFs), with enhanced performance. The removal of hemicellulose and lignin gives rise to a new class of fibers, cellulose microfibers, with enhanced performance. Several authors have worked on the micro/nanofiber-reinforced polymer composites. Savadekar and Mhaske [1] investigated the effect of cellulose nanofibers on the mechanical properties of thermoplastic starch composites. The nanofibers were found to have a positive effect on the mechanical properties of the composites at lower fiber loadings. Huang and Netravali [2] developed the biodegradable composites using bamboo micro/nanofibrils and chemically modified soy protein resin. The incorporation of the microbamboo fibrils increased the fracture stress resistance and the modulus of the soy protein concrete. Silviya et al. [3] investigated the enhanced mechanical and thermal properties of cellulose whisker-loaded poly(ethylene-co-vinyl acetate) (EVA) composites. Bipinbal et al. [4] studied the effect of CMF extracted from coir fiber through chemical and mechanical treatment by incorporating the CMF into natural rubber by latex stage compounding. The mechanical properties of the composites have increased through CMF loading. Kaushik et al. [5] analyzed the properties of celluloses nanofibril/thermoplastic starch-based nanocomposites. Nanofibers were obtained from wheat straw by the steam explosion technique. Celluloses nanofibril/thermoplastic starch showed enhanced mechanical, thermal, and barrier properties. CMF has been observed to be an excellent reinforcement in EVA [6].

The above pioneering research groups are working in micro/nanofiber-reinforced polymer composites with the

---

***Corresponding author: Keereyadath Priya Dasan,** Material Chemistry Division, SAS, VIT University, Tamil Nadu 632014, India, e-mail: priyajeenetd@gmail.com
Ashok Kumar Sonia: Material Chemistry Division, SAS, VIT University, Tamil Nadu 632014, India

aim of improving the mechanical, thermal, and biodegradation behavior of the composites. However, the effect of the microfibers on the microbial growth on composite films when kept in contact with food material is not known.

EVA is a copolymer made up of two constituent homopolymers, that is, polyethylene and poly(vinyl acetate). EVA is one of the most widely used high-barrier family of materials due to its low permeability to gases and organic vapors [7]. It is used in producing film for greenhouses covering [8], food packaging applications [9], and controlled drug release [10]. EVA is characterized by low friction coefficients, better flexibility, heat seal ability, and high adhesivity. EVA has been used in food packaging as shrink wrappers, ice bags, and stretch wrap for refrigerated meat and poultry [11]. The wide use of EVA in food packaging is mainly due to its unique characteristic such as non-toxicity, better seal ability at lower temperature, better film stretch properties, better clarity, and is a very good barrier material to gas and vapors. Because of its excellent adhesion and ease of processing, EVA is used in extrusion coating and in heat sealing layers with PET, cellophane, and biaxially oriented PP films for cheese wrap and medical packages.

Hibiscus sabdariffa is an herbaceous annual plant that is grown commercially in a variety of weather conditions in the southern regions of India. Its leaves are edible and the stem is used for making ropes. The aim of the present work was to study the effect of CMF on the microbial growth in milk, curd, and orange juice when used as filler in polymer composites. The experiments were carried out at three different temperatures for 16 days.

2 Materials and methods

2.1 Materials

Hibiscus sabdariffa fiber was collected from a local farming community. The CMFs were extracted from *H. sabdariffa* by steam explosion, as mentioned in our earlier work [12]. The chemicals used such as NaOH, acetic acid, oxalic acid (SD Fine, Chennai, India), sodium hypochlorite (Rankem, Chennai, India) were reagent grade. Potato dextrose agar and nutrient agar were from Difco Laboratories (Detroit, MI, USA). EVA (18% vinyl acetate content) was obtained from Polyolefin Industries (Chennai, India). Pasteurized milk and curd were purchased from the local market and transported to the laboratory immediately (Aavin, Tamil Nadu Co-operative Milk Producers' Federation). Orange juice was obtained by grinding peeled fresh oranges followed by filtering the pulped juice using linen cloth.

2.2 Preparation of film

The mixing and extrusion of EVA with different filler ratio was carried out in a twin-screw extruder at 150°C using a mixing speed of 60 rpm. The mixes were passed through rollers to obtain a uniform film of thickness 0.5 mm (±0.02 mm). The composites were prepared with different weight percentages of CMF (1, 2.5, 5, 7.5, 10, 12.5, and 15 wt%).

2.3 Evaluation of microbial growth

Food material (50 ml) was poured into 100-ml glass screw cap containers. Circular disc of composite (diameter 1.9 cm) samples were immersed in the food material and the bottle was capped (Figure 1). The cell was than stored at 3°C, 10°C, and 20°C for 16 days. Total aerobic bacteria were counted in colony forming units (cfu). During the storage period, 0.1 ml of food material was taken out periodically to measure microbial change in the stored milk, curd, and orange juice. A series of decimal dilutions were carried out with sterilized distilled water. To enumerate the total aerobic bacterial counts, the serially diluted samples were plated on nutrient agar and incubated aerobically at 36°C for 2 days.

2.4 Modeling of the microbial growth

The microbial growth in milk, curd, and orange juice for aerobic bacteria were further analyzed using Baranyi's growth function [13]:

$$y = y_0 + \frac{\mu}{\ln(10)} \times A - \frac{1}{\ln(10)} \times \ln\left(1 + \frac{e^{\mu A} - 1}{10(y_{\max} - y_0)}\right), \tag{1}$$

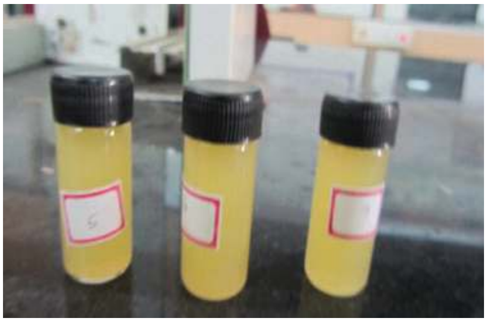

Figure 1 Photographs of microbial growth testing.

where y is the microbial count in $\log_{10}$ (cfu/ml) at time t (day), y_0 and $y_{\max}$ are the initial and final cell numbers in $\log_{10}$ (cfu/ml), respectively, μ is the specific growth rate (per day), and A is defined by the following equation:

$$A=t+\frac{1}{\mu}\times\ln\frac{(1+e^{-\mu t}-1)}{10(y_{\max}-y_0)}, \tag{2}$$

with q_0 being a parameter for the initial physiological state of the microbe cells. The q_0 is related to lag time:

$$t_{\text{lag}}=\frac{\ln(1\text{-}t1/q_0)}{\mu}. \tag{3}$$

The above-mentioned equations (1–3) for the following parameters ($y_{\max}$, μ, and t_{lag}) were determined to describe the microbial growth kinetics on the foods.

3 Results and discussion

3.1 Microbial growth in milk, curd, and orange juice after exposing to EVA/CMF composites

Figure 2A–C shows the number of colonies with days in milk kept in contact with EVA/CMF composites. A gradual growth of microbes or colonies with days is observed in all samples. The presence of microbes on the 0th day may be due to the contamination in the liquid food material during sample preparation. No systematic trend has been observed in microbial growth with CMF loading. The incorporation of filler was found to have negligible influence on the microbial growth in milk at all temperatures. The presence of hydrophilic fibers is likely to allow microorganisms to access the interior of hydrophobic matrix using solvent as a medium [14–16]. However, such a trend is not observed in the present case. One of the reasons may be the low weight percentage of CMF in the composite. Another reason may be the increased barrier nature of composite with fiber incorporation, which does not expose the composite constituents to the solvents/microorganism [17]. Natural fiber consists of long thread-like bundles of microfibrils surrounded by an amorphous phase of hemicelluloses and lignin [18–20]. This leads to high accessible volume and absorption capacity toward solvents. However, a microfiber with a lower diameter and low porous nature is a high-barrier material and decreases the exposure to the solvents or microorganisms.

Colony growth is found to increase with temperature. Generally, an increase in temperature leads to an increase in microbial growth [21]. At a higher temperature, the diffusion of solvents into the polymer matrix increases [22–24]. This results in increased flexibility of polymer chains, allowing more solvents into the matrix, thereby exposing the solvents to higher amounts of CMF. Figures 3A–C and 4A–C represent the colony growth in curd and orange juice

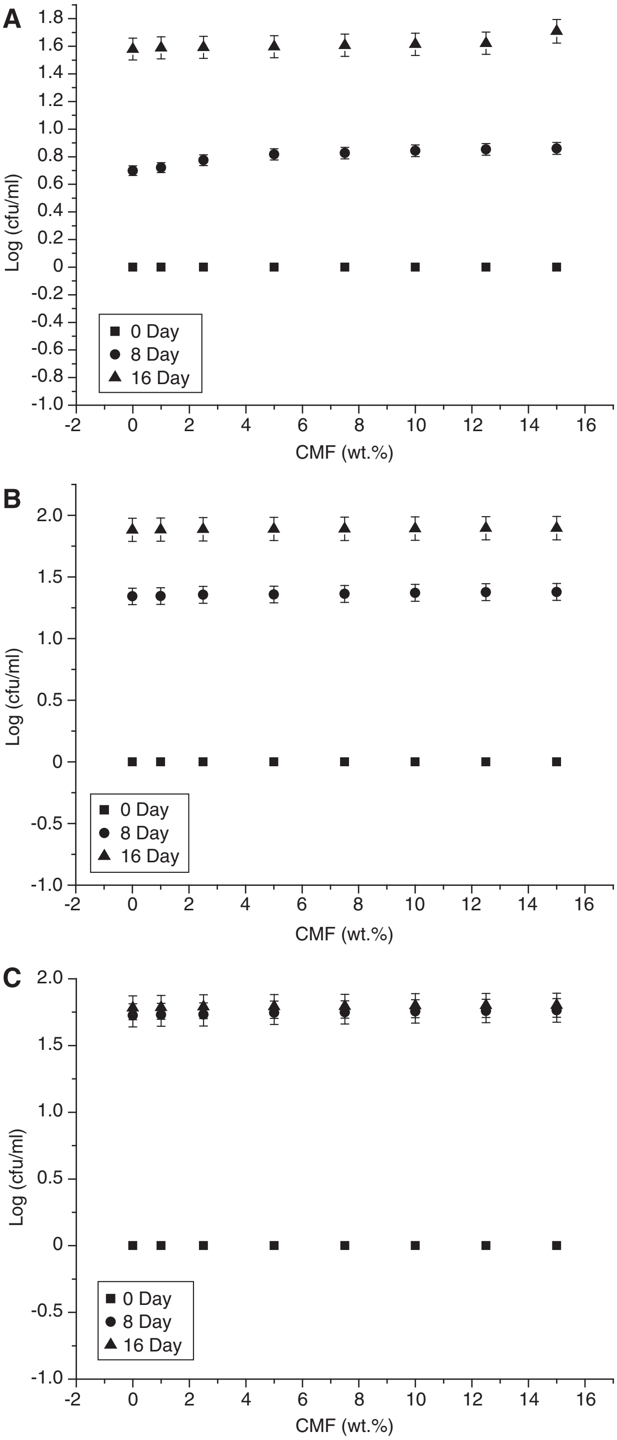

Figure 2 Growth of the microbes in milk when in contact with EVA/CMF composites at (A) 3°C, (B) 10°C, and (C) 20°C.

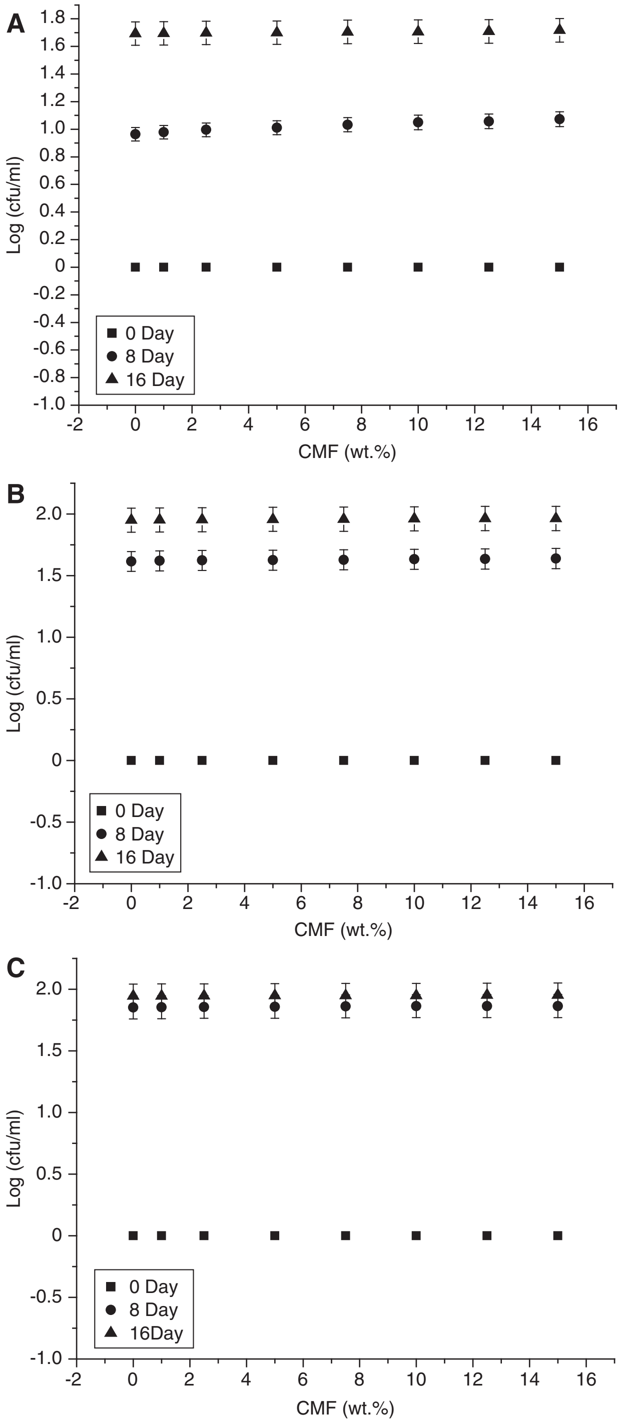

Figure 3 Growth of the microbes in curd when is in contact with EVA/CMF composites at (A) 3°C, (B) 10°C, and (C) 20°C.

at three different temperatures. The same trend as that in milk is observed here. Among milk, curd, and orange juice, colony growth is found to be lesser in orange juice followed by milk and then curd, as shown in Figures 5–7. A minimal or negligible colony growth has been observed in the case of orange juice.

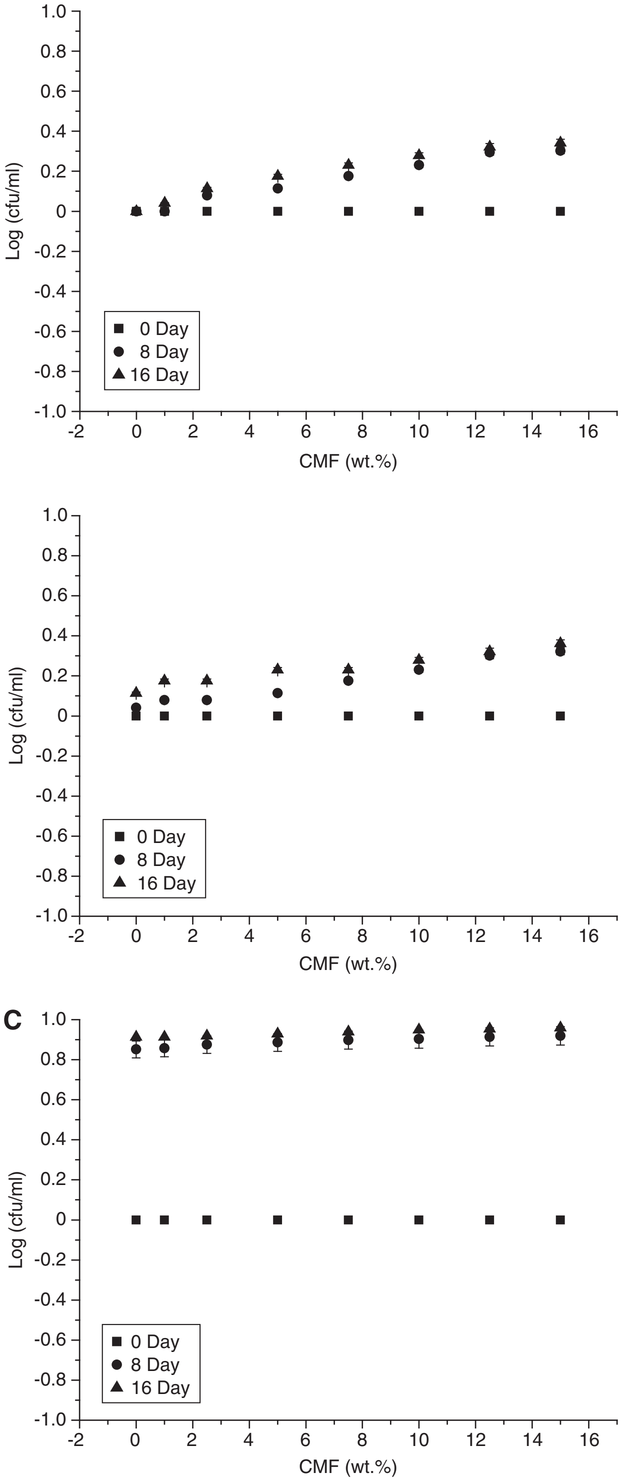

Figure 4 Growth of the microbes in orange juice when is in contact with EVA/CMF composites at (A) 3°C, (B) 10°C, and (C) 20°C.

3.2 Modeling of the microbial growth in milk, curd, and orange juice

Baranyi's growth model parameters such as y_{max}, t_{lag}, and μ factors for EVA/CMF composites are given in Table 1.

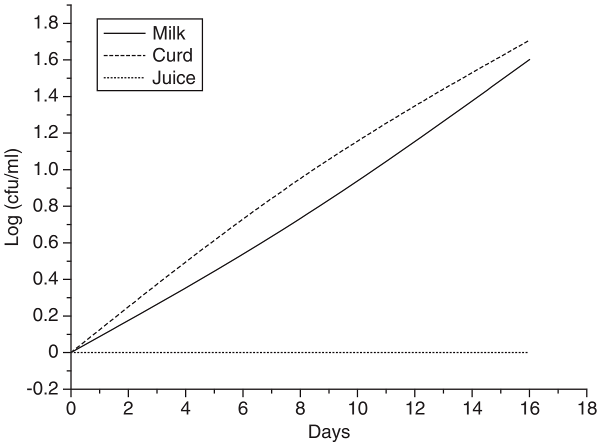

Figure 5 Growth of the microbes in milk, curd, and orange juice when is in contact with EVA/CMF composites at 3°C.

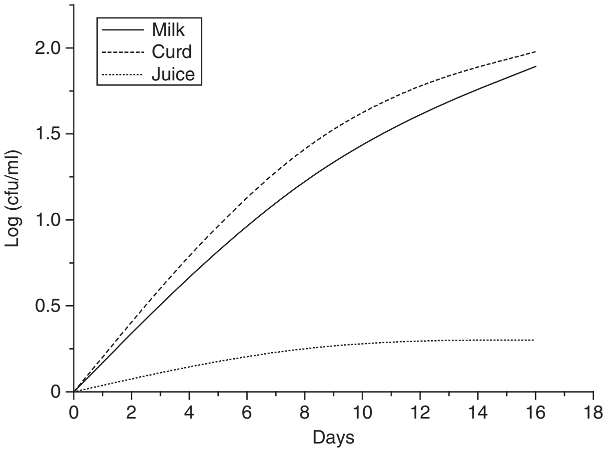

Figure 6 Growth of the microbes in milk, curd, and orange juice when is in contact with EVA/CMF composites at 10°C.

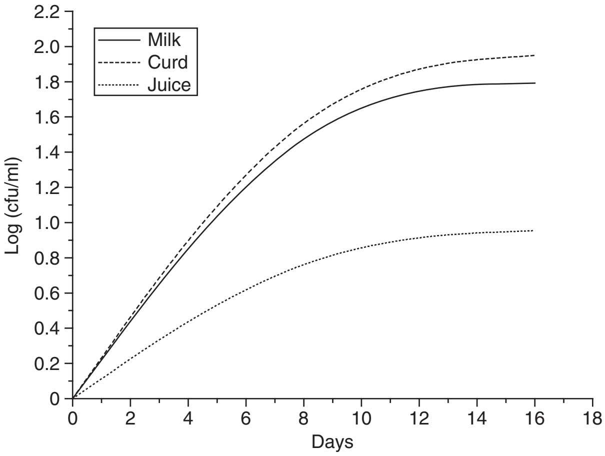

Figure 7 Growth of the microbes in milk, curd, and orange juice when is in contact with EVA/CMF composites at 20°C.

Table 1 Parameters estimated from Baranyi's microbial growth model for total bacteria in milk, curd, and orange juice.

Food type	Storage temperature (°C)	CMF (wt%)	Parameters			
			y_{max}	t_{lag} (day)[1]	μ (day^{-1})	R^2
Milk	3	0	5	0.163	4.37	0.914
		2.5	5.15	0.157	4.75	0.924
		5	5.19	0.164	5.16	0.909
		7.5	5.71	0.162	5.11	0.871
		10	6.00	0.153	4.97	0.741
	10	0	22	0.392	21.22	0.947
		2.5	22.15	0.138	21.45	0.874
		5	22.31	0.113	21.41	0.818
		7.5	22.74	0.164	21.64	0.874
		10	23.01	0.131	21.51	0.912
	20	0	53.07	0.117	52.2	0.874
		2.5	53.12	0.114	52.8	0.876
		5	53.25	0.0889	54.25	0.947
		7.5	53.79	0.0949	54.4	0.915
		10	54	0.0913	55.1	0.907
Curd	3	0	9.2	0.197	8.2	0.997
		2.5	9.45	0.272	8.74	0.967
		5	9.74	0.247	9.941	0.947
		7.5	10.07	0.154	9.28	0.914
		10	10.12	0.347	8.97	0.907
	10	0	41.2	0.101	39.98	0.879
		2.5	41.5	0.0946	40.87	0.817
		5	41.75	0.0935	40.79	0.841
		7.5	41.85	0.0945	41	0.874
		10	42.01	0.0914	39.91	0.907
	20	0	71.2	0.0741	69.87	0.741
		2.5	71.57	0.0724	70.67	0.724
		5	71.77	0.0714	70.68	0.817
		7.5	71.84	0.0698	70.98	0.847
		10	72.07	0.0598	70.4	0.874
Juice	3	0	1	0.834	1	0.901
		2.5	1.2	0.839	1.97	0.914
		5	1.3	0.871	1.1	0.927
		7.5	1.5	0.903	1.3	0.934
		10	1.7	0.879	1.43	0.942
	10	0	1.1	0.889	1.1	0.922
		2.5	1.2	0.874	1.87	0.937
		5	1.3	0.899	1.1	0.927
		7.5	1.5	0.874	1.28	0.937
		10	1.7	0.865	1.54	0.942
	20	0	7.1	0.878	7	0.914
		2.5	7.5	0.859	7.3	0.899
		5	7.7	0.847	7.5	0.748
		7.5	7.9	0.547	7.58	0.871
		10	8	0.647	7.14	0.804

It shows the trend of increasing in microbial counts during storage of milk, curd and orange juice. As discussed above, the maximum cell concentration is found to be the same or there is no significant variation with CMF incorporation and loading. The lag times and growth rate were not dependent on CMF loading. However, colony growth, as well as $y_{\max}$ and μ, is found to increase with increasing temperature.

4 Conclusion

The possibility of CMF/EVA composites for food packaging applications was analyzed at three different temperatures. The presence of CMF does not influence the growth of microbes in food materials. The same trend has been observed at all temperatures irrespective of the food material used. Among the different food materials used, microbial growth was higher in curd followed by milk and then orange juice. The lower microbial presence in orange juice may be due to the acidic nature of orange. The calculations carried out using the Baranyi model rule have been found to complement the experimental results.

References

[1] Savadekar NR, Mhaske ST. *Carbohyd. Polym.* 2012, 89, 146–151.
[2] Huang X, Netravali A. *Compos. Sci. Technol.* 2009, 69, 1009–1015.
[3] Silviya EK, Unnikrishnan G, Varghese S, Guthrie JT. *J. Appl. Polym. Sci.* 2012, 125, 786–792.
[4] Bipinbal PK, Joseph MJ, Sunil KN Kutty. *Adv. Polym. Technol.* 2010, 300–308.
[5] Kaushik A, Singh M, Verma G. *Carbohyd. Polym.* 2010, 82, 337–345.
[6] Sonia A, Priya Dasan K. *Chem. Eng. J.* 2013, 228, 1214–1222.
[7] Lagaron JM, Catala R, Gavara R. *Mater. Sci. Technol.* 2004, 20, 1.
[8] Scaffaro R, Morreale M, Lo Re G, La Mantia FP. *Polym. Degrad. Stab.* 2009, 94, 1220–1229.
[9] Kirwan MJ, Strawbridge JW. In Food Packaging Technology, Blackwell Publishing: London, 2003, pp. 174–240.
[10] Arnold RR, Wei HH, Simmons E, Tallury P, Barrow DA, Kalachandra S. *J. Biomed. Mater. Res.* 2008, 86, 506–513.
[11] Modern Plastics World Encyclopaedia Handbook, McGraw-Hill: New York, 2000, A-193.
[12] Sonia A, Priya Dasan K. *Carbohydr. Polym.* 2013, 92, 668–694.
[13] Baranyi J, Roberts TA. *Food Microb*. 1994, 23, 277–294.
[14] Tudorachi N, Cascaval CN, Rusu M. *Polym. Test.* 2000, 19, 785–799.
[15] Calil MR, Gaboardi F, Guedes CGF, Rosa DS. *Polym. Test.* 2006, 25, 597–604.
[16] Kim H-S, Kim H-J, Lee J-W, Choi I-G. *Polym. Degrad. Stab.* 2006, 91, 1117–1127.
[17] Sonia A, Priya Dasan K. *Food. Eng.* 2013, 118, 78–89.
[18] Kaushik A, Singh M. *Carbohydr. Res.* 2011, 346, 76–85.
[19] Zuluaga R, Putaux JL, Restrepo A, Mondragon I, Ganan P. *Cellulose* 2007, 14, 585–592.
[20] Wang B, Sain M, Oksman K. *J. Appl. Compos. Mater.* 2007, 14, 89–103.
[21] Lee CH, Park HJ, Lee DS. *Food Eng.* 2004, 65, 527–531.
[22] Priya Dasan K, Unnikrishnan G, Purushothaman E. *eXPRESS Polym. Lett.* 2008, 2, 382–390.
[23] Njoku RE, Obikwelu DON. *J. Niger. Eur. Technol.* 2008, 27, 58–63.
[24] Sujith A, Unnikrishnan G. *J. Mater. Sci.* 2005, 40, 4625–4640.