

Optimization of Etherification Reactions for Recycling of Tea Fungal Biomass Waste into Carboxymethylcellulose



Iragavarapu Akhil Gargey, Dash Indira, R. Jayabalan
and P. Balasubramanian

1 Introduction

Cellulose ethers are industrially world's most produced ethers, and the primary source of raw material for cellulose is obtained mainly from plants. Cellulose is a polymer made of glucose monomer units. It is located in plants cell wall. Industrially, extraction of cellulose is highly tricky and expensive because it is tightly bound to hemicellulose and lignin polymer chains. Large areas of fertile lands are wasted in growing these plants to obtain cellulose. These lands are continuously reused for plantation. Microorganisms are also a significant source for production of cellulose; they take sucrose, glucose, and mannose as substrates and excrete cellulose as a waste product. Incubation periods of these microorganisms vary from 24 h to 1 week. These organisms are used industrially to produce cellulose as they overcome the difficulties faced while processing cellulose from plants. Industrially, cellulose is obtained from plants or microorganisms and converted into carboxymethylcellulose (CMC) by etherification reaction [1]. CMC is a cellulose derivative with carboxymethyl groups ($-\text{CH}_2-\text{COOH}$) bound to some of the hydroxyl groups of the glucopyranose monomers that make up the cellulose backbone. It is often used as its sodium salt, sodium carboxymethylcellulose.

CMC is the most broadly utilized cellulose ether today, with applications in the cleanser, paper material, pharmaceutical, and paint businesses. Manufacturing of CMC is less severe than that of most other cellulose ethers since all responses are worked at atmospheric pressure and economically available reagents. The etheri-

I. A. Gargey · P. Balasubramanian (✉)
Department of Biotechnology and Medical Engineering,
NIT Rourkela, Rourkela, Odisha, India
e-mail: balap@nitrkl.ac.in

D. Indira · R. Jayabalan (✉)
Department of Life Science, NIT Rourkela, Rourkela, Odisha, India
e-mail: jayabalanr@nitrkl.ac.in

fying reagent, sodium monochloroacetate, is easy to handle and exceptionally proficient. Therefore, CMC has turned into the biggest mechanical cellulose ether. Expansive amounts are delivered in unrefined business grades with no refining for use in cleansers, oil boring, and in the paper business. High-virtue evaluations are additionally utilized as sustenance added substances [2].

Kombucha tea is devoured worldwide for its invigorating and useful properties on human well-being. Reports asserting that drinking Kombucha can avert different sorts of malignancy and cardiovascular illnesses, promote liver capacities, and fortify the immune system. Kombucha tea (symbiotic association of yeast and bacteria) produces abundant mats (fungal tea biomass) rich in cellulose as a waste product [3]. This biomass waste can be used to produce cellulose ether (carboxymethylcellulose, CMC) by simple chemical conversion called Williamson's ether synthesis [4, 5].

The present paper aimed to evaluate the influencing factors on conversion of Kombucha tea waste into carboxymethylcellulose and optimizing the etherification reaction conditions to achieve the maximal yield of carboxymethylcellulose.

2 Materials and Methods

2.1 *Preparation of Kombucha Tea*

About 50 g of sucrose was weighed and added to the boiling water and stirred for 5 min. Then, 6 g of tea leaves/powder (green tea leaves, black tea powder, and tea waste material) was added and stirred for 5 min to make a decoction. The mixture was filtered and distributed among 15 flasks, five flasks containing one brewed tea. Each flask contained 100 ml of brewed tea. Previously inoculated culture (3 g) was added along with 10 ml of previously fermented tea liquor which reduces the pH thereby preventing the growth of contaminants. The flasks were covered with paper cloth to keep insects away. The preparation was incubated at 20 to 22 °C (the optimal incubation temperature ranges from 18 to 26 °C) in dark and away from sunlight [6].

2.2 *Estimation of Various Compounds in Tea Broth as the Fermentation Proceeds Over Time*

Sampling was done every third day of fermentation. Tea broth sample (5 ml) was taken from each flask after removing the mats and stored at 4 °C until analysis. HPLC was used to determine various compounds present in the broth. The concentration of biomolecules in Kombucha during fermentation was estimated using high-performance liquid chromatographic system with refractive index detector (Shimadzu, Japan). Agilent Hiplex column was used with 5-mM sulfuric acid as

mobile phase, 0.7 mL/min as flow rate, and 60 °C as column temperature. Amount of metabolites were estimated by using respective standards purchased from HiMedia, Mumbai, India, and Sigma, USA. Citric acid, sucrose, glucose, acetic acid, glycerol, and ethanol estimations were done during the 14-day period for all three tea broths.

2.3 Conversion of Tea Fungal Biomass Waste into Carboxymethylcellulose

Biomass waste (1 g) was taken in 250-ml bottle. Then, 20-ml IPA is added to the biomass powder and stirred for 5 min. The bottle was placed on a magnetic stirrer at 200 rpm, and 2 ml of 30% NaOH was added dropwise using a micropipette and stirred for an hour. The mixture was filtered, and the residue was suspended in 60-ml methanol overnight. Residue was again filtered and dried to constant weight [7]. The obtained product (as shown in Fig. 1) was subjected to Fourier transform infrared spectroscopic (FTIR) analysis for further characterization and to determine the degree of substitution.

2.4 Determination of Degree of Substitution (DS)

Etherified samples (0.2 mg) were pelletized with KBr and were finely ground and pressed. The infrared spectra of these samples were recorded with a Perkin Elmer spectrometer between 400 and 4000 cm^{-1} . The degree of substitution of the carboxyl group in CMC can be determined by taking the ratio of the absorption spectra by following Eq. (1).

$$R_{\text{rel}} = \frac{A_{1605}}{A_{2920}} \quad (1)$$



Fig. 1 Reaction scheme of the carboxymethylation of cellulose

Table 1 Experimental range of influencing factors on etherification of tea fungal biomass waste to carboxymethyl cellulose

Factors	Name	Units	Low	High
A	NaOH reaction time	Minutes (min)	30	180
B	SMCA concentration	Grams (g)	0.2	1
C	NaOH concentration	% (w/v)	10	50
D	IPA concentration	% (v/v)	50	100
E	SMCA reaction time	Minutes (min)	30	180

The relative degree of substitution (DS_{rel}) can be determined by following Eq. (2) [8].

$$DS_{rel} = R_{rel} - 1 \quad (2)$$

The above equations were utilized to estimate the relative amount of carboxyl groups in the recycled tea waste samples.

2.5 Optimization of Reaction Parameters

Biomass waste (0.5 g) was taken in 250-ml Schott bottle. Then, 10 ml of absolute IPA was added and stirred for 5 min. One ml of 30% (w/v) NaOH was added dropwise and stirred for an hour on a hot plate magnetic stirrer. After that, 0.6 g SMCA was added and stirred for three hours on a hot plate magnetic stirrer at nearly 200 rpm and 45 °C. Mixture was filtered and suspended in 30-ml methanol overnight. Residue was again filtered and dried up to constant weight. This was repeated five times, each time varying a parameter and keeping all other parameters constant to follow a one variable at a time approach. Concentration of IPA was varied from 50 to 100% (v/v). Weight of SMCA was varied from 0.2 to 1 g. Reaction periods of both NaOH and SMCA were varied from range 30 to 180 min. Concentration of NaOH was varied over a range of 10 to 50% (w/v). The obtained products after etherification reaction from tea fungal biomass waste were subjected to FTIR analysis to determine the degree of substitutions. Table 1 outlined the range of influencing factors on experimental conditions.

3 Results and Discussion

3.1 Optimum Tea Broth for Production of Maximum Biomass

It was evident from Fig. 2 that green tea supports the maximum growth rate of tea fungus, and hence, the production of higher biomass waste compared to other two

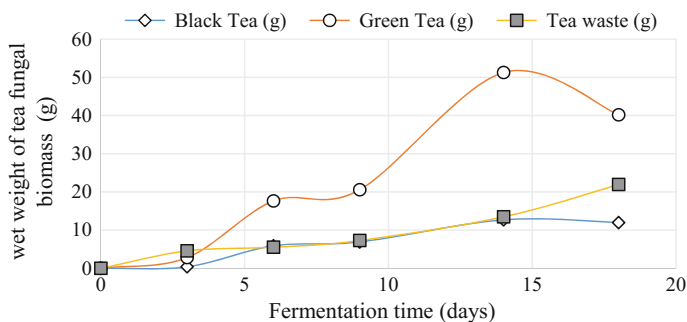


Fig. 2 Growth trend of fungal biomass on different tea substrates

tea substrates was studied. Black tea and tea waste material showed an increase in weight of fungal tea biomass up to 14 days. Tea waste material supported an increase in fungal tea biomass even after 18 days. All the tea substrates showed an increase in biomass for 14 days, and there was a slight decrease in the biomass after 14 days in green tea and black tea due to the two following reasons. Firstly, after 14 days of fermentation bacteria yeast have entered into stationary phase and the pH was decreased which led to the death of acid-sensitive cells in the biomass. Acid-sensitive microbial cells were released into the tea broth which would have decreased the weight of the fungal tea biomass. Secondly, all the collected tea fungal biomasses were blotted on a filter paper for 5 min. The rate of absorption of water from these biomasses was proportional to their wet weights. Hence, more amount of water was absorbed from biomasses which were collected on day 18. This led to the decrease in weights considerably compared to biomasses taken on day 14.

3.2 Proof of Carboxymethylation

As shown in Fig. 3, peaks at 1420 and 1320 cm^{-1} were observed in the absorbance spectra of recycled tea fungal biomass waste to carboxymethylcellulose product obtained from the etherification reaction. These peaks correspond to CH_2 scissoring and $-\text{OH}$ bending vibration, respectively. Successful conversion of fungal tea biomass into CMC was indicated by the presence of peaks at 2920 and 1605 cm^{-1} which correspond to carboxymethyl group and $\text{C}-\text{H}$ vibration, respectively. The conversion of cellulose to CMC was validated by comparing the spectra obtained from the tea biomass to that of commercially available CMC as shown in Fig. 4.

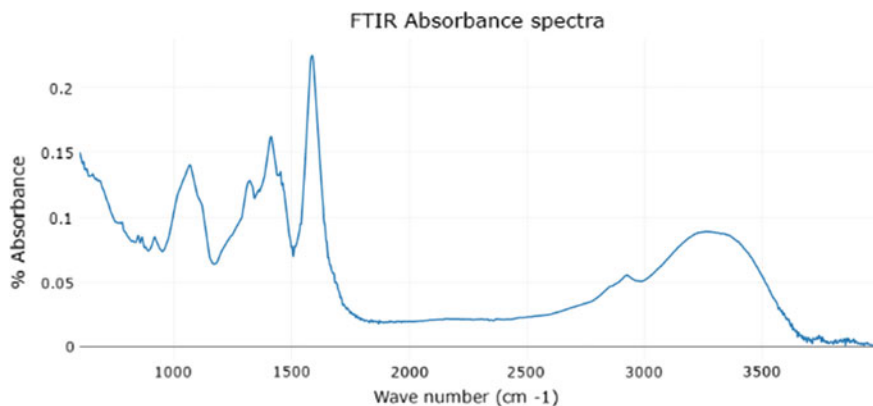


Fig. 3 FTIR spectrum of carboxymethylcellulose obtained from fungal tea biomass through etherification reaction

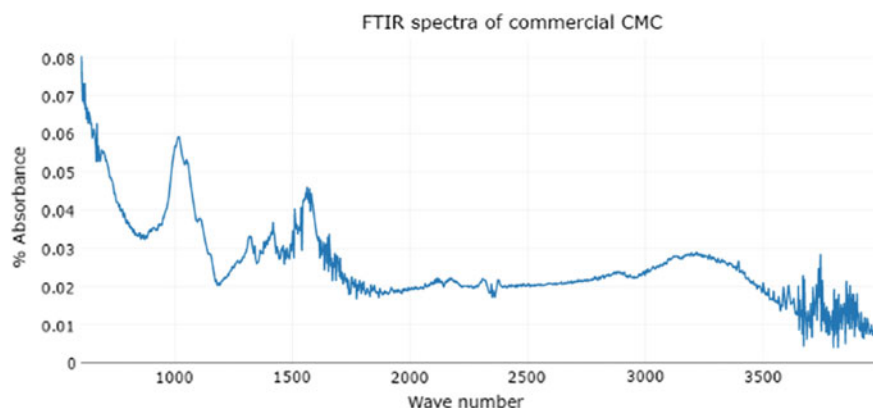


Fig. 4 FTIR spectrum of commercially available carboxymethyl cellulose

3.3 *Effects of Variation of Parameters on the Degree of Substitution*

3.3.1 **Effect of Reaction Period of NaOH**

As shown in Fig. 5, the carboxymethylation reaction was carried out in six different reaction periods ranging from 30 to 180 min. A maximum DS of 2.73 was obtained with 120 min. There was an increase of DS with reaction period up to 120 min and after that DS decreases. This increase may be because there is better reaction

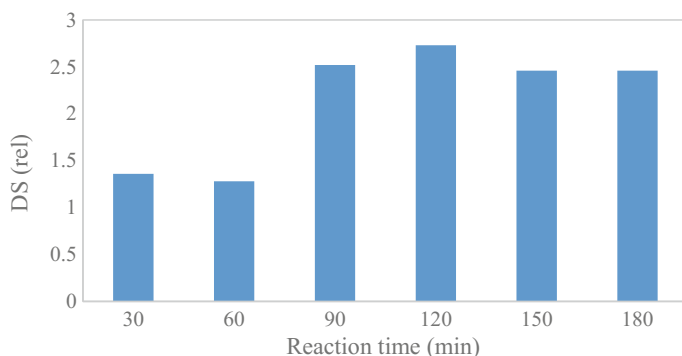


Fig. 5 Effect of reaction period of NaOH on degree of substitution of CMC from fungal tea biomass

environment created due to the prolonged duration of reaction [9]. This helps in diffusion and absorption of reactants to make better contact with each other. A possible explanation for the decrease after 120 min may be due to an increased degradation of the polymer.

3.3.2 Effect of Concentration of Isopropyl Alcohol

As shown in Fig. 6, the carboxymethylation reaction was carried out in three water: isopropyl alcohol concentrations. A maximum DS of 2.28 was obtained at 10:90 (v/v %). It was observed that increase in the rate of organic solvent increases the DS of the CMC produced. It was observed that isopropyl alcohol causes pronounced decrystallization of cellulose and a change of polymorphism from cellulose I to cellulose II.

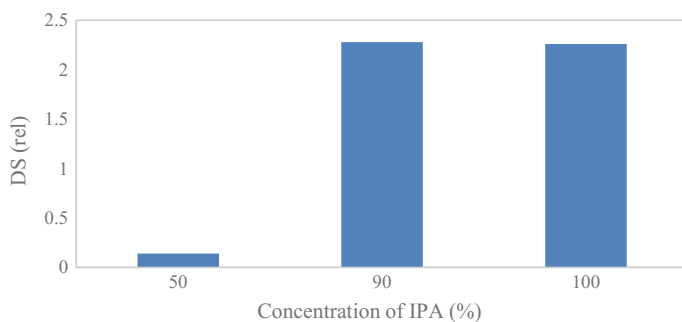


Fig. 6 Effect of various concentrations of IPA on degree of substitution of CMC from fungal tea biomass

The change of crystallinity may be due to the partition of sodium hydroxide between cellulose and reaction medium. This occurs while cellulose chains were suspended in a mixture of organic solvent, water, and sodium hydroxide. The decrease in DS after 10:90 (v/v %) may be attributed to the fact that isopropyl has a high dielectric constant and is a harmful solvent for sodium hydroxide in the presence of cellulose.

3.3.3 Effect of Concentration of SMCA

As shown in Fig. 7, the carboxymethylation reaction was carried out in five different concentrations of sodium monochloroacetate. A maximum DS of 2.9 was obtained at 1 g SMCA concentration. There was an increase in DS concentration of SMCA. The increase probably is due to higher availability of acetate ions at a higher concentration in the proximity of cellulose molecules.

3.3.4 Effect of concentration of NaOH

As shown in Fig. 8, the effect of sodium hydroxide concentration was studied by varying the concentration of the sodium hydroxide solution. It was observed that DS of CMC increased with NaOH concentration and attained a maximum DS of 3.54 at 40% (w/v) concentration. After that, the decline in DS may be due to the side reaction of sodium hydroxide and SMCA producing sodium glycolate. Predomination of this side reaction decreases SMCA concentration, and thereby, it directly affects the degree of substitution [10].

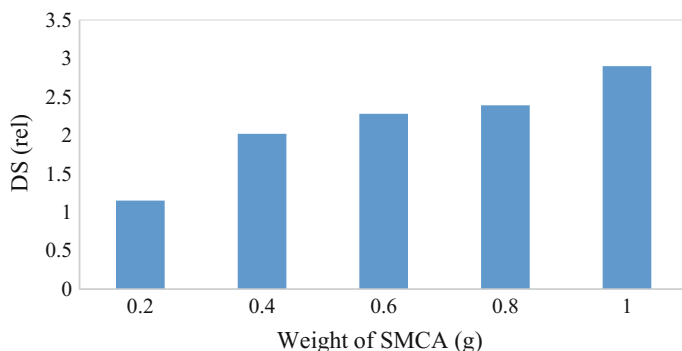


Fig. 7 Effect of various concentrations of NaOH on degree of substitution of CMC from fungal tea biomass

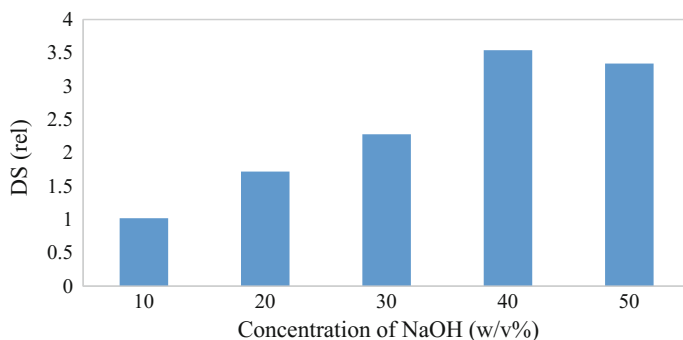


Fig. 8 Effect of various concentrations of NaOH on degree of substitution of CMC from fungal tea biomass

3.3.5 Effect of Reaction of SMCA

As shown in Fig. 9, carboxymethylation reaction was carried out in five different reaction periods. A maximum DS of 2.5 was obtained with 150 min. There was an increase in DS with reaction period up to 150 min and after that DS decreases. The increase may be due to the fact that prolonged duration of reaction allows better contact of reactants. The decline after 150 min is due to decrease in reaction efficiency. This was explained by Pusphamalar et al. [7] who carried similar studies on sago pulp waste.

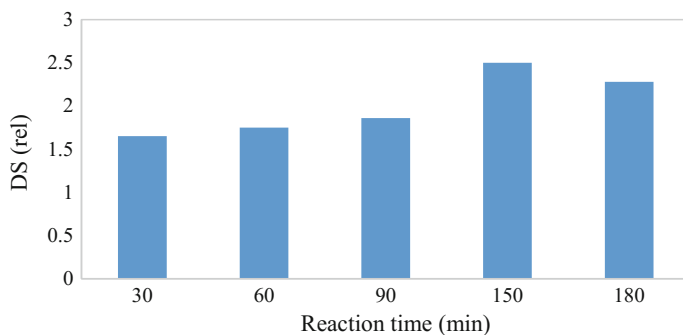


Fig. 9 Effect of various reaction periods of SMCA on degree of substitution of CMC from fungal tea biomass

4 Conclusion

The present paper evaluated the possibilities of converting tea fungal biomass waste into the useful product such as carboxymethylcellulose through etherification reactions. The influencing factors of the etherification reaction such as the concentration of SMCA, isopropyl alcohol, NaOH, and the reaction time of SMCA and NaOH were optimized using one variable at a time approach. The optimal conditions for the maximal yield of carboxymethylcellulose from cellulose obtained from tea fungal biomass waste were as follows: NaOH reaction period of 120 min; SMCA weight of 1 g; NaOH concentration of 40%; IPA concentration of 90%; SMCA reaction period of 150 min. These optimized conditions could be helpful while utilizing the tea fungal biomass waste to carboxymethylcellulose during the scale-up waste to wealth process for attaining sustainability.

References

1. Xiquan, L., Tingzhu, Q., Shaoqi, Q.: Kinetics of the carboxymethyl cellulose in the isopropyl alcohol system. *Acta Polym.* **41**, 220 (1990)
2. Dahlman, O., Jacobs, A., Sjöberg, J.: Molecular properties of hemicelluloses located in the surface and inner layers of hardwood and softwood pulps. *Cellulose* 325–334 (2003)
3. Jayabalan, R., Malabasa, R., Satishkumar, M.: Kombucha tea: metabolites. In: *Fungal Metabolites* (2015)
4. Tijssen, C.J., Kolk, H.J., Stamhuis, E.J., Beenackers, A.A.C.M.: An experimental study on the carboxymethylation of granular potato starch in non-aqueous media. *Carbohydr. Polym.* **45**, 219–226 (2001)
5. Khiari, R., Salon, M.C.B., Mhenni, M.F., Mauret, E., Belgacem, M.N.: Synthesis and characterization of cellulose carbonate using greenchemistry: Surface modification of Avicel. *Carbohydr. Polym.* **163**, 254–260 (2017)
6. Jayabalan, R., Radomir, M.V., Satishkumar, M., Jasmina, V.S.: A review on Kombucha tea—microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Compr. Rev. Food Sci. Food Saftey* **13**, 538–550 (2014)
7. Pusphamalar, V., Langford, S., Ahmad, M., Lim, Y.: Optimization of reaction conditions for preparing carboxymethyl cellulose from sago waste. *Carbohydr. Polym.* **64**, 312–318 (2006)
8. Miyamoto, K., et al.: Preparation of carboxymethyl-gellan. *Carbohydr. Polym.* **30**, 161–164 (1996)
9. Bhattacharyya, D., Singhal, R.S., Kulkarni, P.R.: A comparative account of conditions for synthesis of sodium carboxymethyl starch from corn and amaranth starch. *Carbohydr. Polym.* **27**, 247–253 (1995)
10. Khalil, M.I., Hasem, A., Habeish, A.: Carboxymethylation of maize starch. *Starch/Stärke* **42**, 62–63 (1990)