Effect of addition of SO_2 on solid-state fermentation of apple pomace

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Addition of up to 150 ppm of sulphur dioxide (SO_2) in solid-state fermentation (SSF) of apple pomace by *Candida utilis* and *Torula utilis*, and 200 ppm in case of *Saccharomyces cerevisiae* generally enhanced the ethanol concentration, fermentation efficiency and decreased the reducing sugars. But 250 ppm and above of SO_2 proved inhibitory to all the yeasts tried. The titrable acidity remained similar except for a small increase in the treatment with 300 ppm of SO_2. The yeast count decreased after 200 ppm of SO_2. Bacterial and fungal contamination was the highest at 50 ppm and decreased after this level. The yeast counts at 150–200 ppm of SO_2 after 96 h of SSF of apple pomace were quite comparable with those obtained in the steam-sterilized treatment prior to inoculation. Addition of 150–200 ppm of SO_2 can be used as a practical alternative to sterilization in solid-state fermentation of apple pomace.

APPLE pomace is a waste from apple juice production and its disposal into the environment is a serious problem[1,2]. Its utilization by solid-state fermentation for ethanol[3,4] production or concomitant production of ethanol and animal feed has been reported[5]. To have effective solid-state fermentation, a more or less microbe-free environment is a prerequisite. There are always possibilities of contamination from atmosphere and container[6]. Compared to steam sterilization, use of chemicals for this purpose will be far cheaper. Preservatives such as potassium metabisulphite, sorbic acid and dimethyldicarbonate (DMDC) can be used as an alternative to pasteurization, filtration and sterilization[7]. In preservation of fruits and vegetable products KMS (providing SO_2) is used extensively[8] and its use in the solid-state fermentation would be cheaper than steam sterilization. Lack of any information on the use of SO_2 in the solid-state fermentation of apple pomace prompted us to carry out this study.

Three yeasts, viz. *Saccharomyces cerevisiae*, *Candida utilis* and *Torula utilis*, were grown in the yeast malt extract broth and used at the rate of 5% to initiate the solid-state fermentation. Apple pomace used was collected from 'hpmc' fruit-processing plant, Parwanoo (HP). Fresh apple pomace was used within 2 h of collection. Potassium metabisulphite (KMS) was added to the apple pomace. Six concentrations of SO_2 ranging from 50 to 300 mg/l (ppm) were tried. Various combinations of apple pomace after addition of SO_2 were not sterilized. A set without SO_2 addition served as a control. Fermentations were carried out in conical flasks. Before inoculation of yeasts in all the flasks and addition of KMS and ammonium sulphate, 1 g of sample from each flask was taken out aseptically for total microbial count, which was termed as 0 h reading. Fermentation was carried out for 4 days. In all the fermentations, the pH and moisture content were kept at the original level[5]. The fermented apple pomace was analysed for ethanol content by the method described by Caputi *et al.*[9]. The total microbial count was determined by the routine pour plate method[10]. Reducing sugars were measured by the DNS method[11], while total sugars were estimated after hydrolysis with acid prior to estimation. Titrable acidity was estimated as per the routine method[12].

Results in Table 1 reveal that in the case of fermentation of apple pomace by *Saccharomyces cerevisiae*, SO_2 concentration up to 200 ppm increased the ethanol production, after which it declined. However, in the case of fermentation by *Candida* and *Torula* ethanol productivity increased up to 150 ppm SO_2 only. In all the cases, 300 ppm of SO_2 proved to be inhibitory as shown by lowest ethanol production. Increasing SO_2 concentration decreased the sugar content in the medium. But the decrease was not exactly proportional to the increase in ethanol produced. Addition of SO_2 might have provided effective aseptic/anaerobic environment,

Table 1. Effect of SO_2 on some physicochemical characteristics of apple pomace fermented by solid-state fermentation

SO_2* (ppm)	Ethanol (% v/v)			Fermentation efficiency (%)			Reducing sugar (%)			Titrable acidity (% MA)		
	Sac	Ca	Tu	Sac	Ca	Tu	Sac	Ca	Tu	Sac	Ca	Tu
0	3 92	3 71	3 93	60	57	60	2 50	2 76	2 45	0.32	0 35	0 32
50	4 11	3 80	3 93	63	58	60	2 24	2 70	2 39	0 35	0 38	0 35
100	4.38	3 99	3 86	67	61	59	2 42	2 62	2 54	0 38	0 42	0 35
150	4 43	4 59	4 10	67	61	59	2 28	2 10	2 40	0 32	0 37	0 26
200	3 98	4 50	3 92	68	49	60	2 26	2 84	2 54	0 32	0 36	0 32
250	3 47	3 21	4 02	53	69	62	2 46	2 34	2 38	0 38	0 38	0 38
300	2 69	3 62	2 42	41	56	37	3 28	3 12	3 24	0 45	0 54	0 54

Sac, *Saccharomyces cerevisiae*; Ca, *Candida utilis*; Tu, *Torula utilis*, MA, Malic acid
*As potassium metabisulphite.

Table 2. Effect of different concentrations of SO_2 on microbial population (CFU/g) after 96 h of solid-state fermentation

	Inoculated with								
	Saccharomyces cerevisiae			*Torula utilis*			*Candida utilis*		
Treatment	Bacteria	Fungi	Yeast	Bacteria	Fungi	Yeast	Bacteria	Fungi	Yeast
Sterilized	—	—	4.5×10^5	—	—	4×10^5	—	—	4×10^5
Control (no SO_2)	4.20×10^5	4×10^4	8×10^4	3.8×10^5	1×10^4	1.1×10^5	2×10^5	2×10^4	4×10^4
50 ppm	2.80×10^5	—	1×10^5	2.5×10^5	2×10^4	2.2×10^5	1.8×10^5	3×10^4	2×10^5
100 ppm	1.80×10^5	2×10^4	1.2×10^5	1.6×10^5	2×10^4	2.4×10^5	1.5×10^5	5×10^4	2.3×10^5
150 ppm	6×10^4	1×10^4	1.4×10^5	7×10^4	2×10^4	4×10^5	1×10^5	—	3×10^5
200 ppm	5×10^4	—	4.8×10^5	6×10^4	—	4.5×10^5	8×10^4	—	3.7×10^5
250 ppm	4×10^4	1×10^4	2.5×10^5	4.5×10^4	—	1.2×10^5	8×10^4	—	2.1×10^5
300 ppm	2×10^4	—	2.1×10^5	3×10^4	—	5×10^4	6×10^4	—	1.3×10^5

0 h Reading (microbial population) = bacteria 9×10^6 and yeast 1×10^5.

increasing the ethanol content, or could have maintained the ethanol level already produced in the medium by preventing its oxidation or its consumption by yeast cell after completion of fermentation, as has also been observed by Kargi *et al.*[13] using reducing agents such as cysteine and sodium thioglycollate in the ethanolic fermentation. Further, as the product of anaerobic mode of growth increased, concentrations of SO_2 higher than 150 ppm might have decreased the growth, and hence the metabolite, i.e. ethanol. The decrease of ethanol content at 300 ppm is clearly the result of toxicity of SO_2 to the yeasts. SO_2 after 200 ppm suppressing the fermentation is also supported by the findings of Terrell *et al.*[7], wherein 0.4 mM of SO_2 used in various combinations with other preservatives suppressed the fermentation. Fermentation of sugar by the yeast results in ethanol production and, to provide optimum conditions, naturally increases the efficiency of the process. It corroborates with the behaviour of the yeasts used in solid state fermentation of apple pomace.

The titrable acidity of the fermenting medium remained almost similar except for a slight increase in the pomace when 300 ppm of SO_2 was added. It indirectly shows that ethanolic fermentation took place satisfactorily in all the combinations except where 300 ppm of SO_2 was added. From the results it is also clear that trend in the acidities was almost similar in all the yeasts.

The results (Table 2) on the effect of different concentrations of SO_2 on microbial growth revealed that there is similarity in the effect of SO_2 on the growth of yeast, bacteria and fungi. It was found that 250 ppm of SO_2 was toxic to the yeasts (*Saccharomyces*, *Torula*, *Candida*) as there is low yeast count after 200 ppm of SO_2. A large number of bacteria and fungi were found in the case of yeast-inoculated sets of fermentation, indicating that complete sterility, as with steam sterilization, is not achieved with SO_2. With the increase in SO_2 concentration the bacterial and fungal count decreased, and the yeast count of the inoculated yeast increased. It is desirable from the ethanolic fermentation point of view. It has been reported[3] that natural fermentation of apple pomace also produces ethanol and addition of SO_2 might have prevented the activity of natural microflora and allowed the activity of yeast for better ethanol yield by solid-state fermentation. Microbial counts showed that the bacterial population was found maximum in the case of 50 ppm SO_2 in all the three inoculations of yeasts, and after 50 ppm there is considerable decrease in bacterial growth. Fungal growth was less than bacterial growth in all the concentrations of SO_2 tried. These findings suggested that the use of SO_2 can be made to achieve satisfactory solid-state fermentation of apple pomace.

Considering the ethanol yield, sugar content, titrable acidity, effect on different yeasts and their fermentation efficiency and microbial population, it is apparent that addition of 150–200 ppm of SO_2 is advantageous. Concentration of SO_2 higher than this may not be preferred due to the nonuniform effect on one or the other yeast in ethanolic fermentation of apple pomace by solid-state fermentation.

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