IRAQI Academic Scientific Journals
العراقية المجلات الأكاديمية العلمية
University of Kut Journal
ISSN (E): 2616 - 7808 II ISSN (P): 2414 - 7419
www.kutcollegejournal.alkutcollege.edu.iq
k.u.c.j.sci@alkutcollege.edu.iq
Special Issue for the Researches of the 6th Int. Sci. Conf. for Creativity for 16 -17 April 2025

Isolation and Characterization of *Serratia Liquifaciences* to Produce Butanol Aerobically

Doaa Oday Ali [1]

Abstract

Serratia liquifaciences, which have the potential to create butanol after degrading cellulose components in animal waste in the presence of Oxegen at 37 ºC, were isolated from animal samples wastes and cultivated on nutritive broth with various concentrations of butanol.

After that, the experiments were carried out using particular media containing various dilutions of animal feces injected with isolated 1 mg/l bacteria Serratia liquifaciences after adjusted pH to 7.0, then results refer to exist high rate of butanol, reaching to 164.6g/l, after incubation up to 10 days, at 37ºC.

Results showed that optical density (OD) for culture after different period of incubation time, were recorded at 540 nm with (0.004, 0.0066, 0.013, 0.014, 0.39 and 0.66), also, using another sample as in paper wastes to test the ability of bacteria to produce butanol. So, the obtained concentrations of glucose were (19, 18.3, 22, 23, 22 and 24) mg /l, respectively, and the obtained butanol by this methods19.7g/l.

Keywords: Cellulose, cellulase, butanol, glucose, Serratia liquifaciences

Affiliation of Author

[1] Higher institute of forensic sciences, Al-Nahrain university, Iraq, Baghdad, 10001

[1] doaalara3334@gmail.com

[1] **Corresponding Author**

Paper Info.

Published: Oct. 2025

عزل وتشخيص بكتريا *Serratia Liquifaciences* لانتاج البيوتانول هوائياً

دعاء عدي علي [1]

المستخلص

عُزلت بكتيريا Serratia liquifaciences، القادرة على إنتاج البيوتانول بعد تحليل مكونات السليلوز في نفايات الحيوانات باستخدام الأوكسجين عند درجة حرارة 37 مئوية، من عينات لنفايات الحيوانات، وزرعت في وسط مغذي بتراكيز مختلفة من البيوتانول. بعد ذلك، أُجريت التجارب باستخدام أوساط خاصة تحتوي على تخافيف مختلفة من براز الحيوانات، وحُقنت ببكتيريا Serratia liquifaciences المعزولة بتركيز 1 ملغم/لتر، بعد تعديل الرقم الهيدروجيني إلى 7.0، حيث أشارت النتائج إلى وجود تركيز عالٍ من البيوتانول، وصل إلى 164.6 غ/لتر، بعد فترة حضانة استمرت 10 أيام عند درجة حرارة 37 مئوية. أظهرت النتائج أن الكثافة الضوئية (OD) للمزارع بعد فترات حضانة مختلفة، سُجلت عند طول موجي 540 نانومتر بقيم (0.004، 0.0066، 0.013، 0.014، 0.39 و0.66)، كما تم استخدام عينة أخرى من نفايات الورق لاختبار قدرة البكتيريا على إنتاج البيوتانول منها. فكانت تراكيز الجلوكوز المتحصل عليها من تحلل الورق (19، 18.3، 22، 23، 22 و24) ملغم/لتر، على التوالي، والبيوتانول المتحصل عليه بهذه الطريقة 19.7 غ/لتر.

الكلمات المفتاحية: سيليلوز، انزيم السيليليز، بيوتانول، كلوكوز، السريشيا

انتساب الباحث

[1] المعهد العالي للعلوم العدلية، جامعة النهرين، العراق، بغداد، 10001

[1] doaalara3334@gmail.com

[1] **المؤلف المراسل**

معلومات البحث

تأريخ النشر: تشرين الاول 2025

Introduction

Cellulolytic microbes play a key role in the biosphere by recycling cellulose, the carbohydrate most commonly produced by plants. Cellulose is a polysaccharide consisting of hundreds to thousands (14) of linear D-glucose units with the formula (C6H10O5)n [1]. The major cell walls of green plants, many algae and oomycetes contain cellulose as a structural component. Biofilms [2]. The most prevalent organic polymer on Earth is cellulose, which is resistant to enzymatic hydrolysis [3]. All organisms known to efficiently digest cellulose produce a set of enzymes with different specificities that work in concert. Cellulolytic enzymes have been studied at the

molecular level and several properties have been discovered that contribute to their action. Despite their diversity, sequence analysis indicates that the catalytic cores of cellulases belong to only a few families. The data suggest that numerous enzymes within each family share similar folding patterns and catalytic residues. It also makes up most of the plant waste. Their use requires the hydrolysis of cellulose and other cell wall polysaccharides into simple sugars which could may be on of their importants in full product [4]. Synthesis of biofuels such as butanol from plant waste (waste paper) or animal waste relies on the action of fungi or bacteria.bacteria (Serratia): is a genus of bacteria of the family Enterobacteriaceae. *Serratia liquefaciens* is a liquefaction bacterium. It should be noted that the author [5] considers this strain to be a typical strain of (Aerobacter liquefaciens) or (Aerobacter lipolyticus).In the intestinal tracts of cows, horses, sheep, goats, and termites, this symbiotic bacteria exist. The enzymes needed to breakdown cellulose in the GI tract are found in these symbiotic bacteria. They have the enzymes needed to break down or hydrolyze cellulose, whereas mammals, including termites, do not have it. Cellulose cannot be digested by any vertebrate [6]. Serratia was carried in the digestive tract of animals, but they were unaffected [7]. "Biobutanol" is a desirable biofuel. Butanol is used in the chemical industry as a fuel, and it helps to clean the air by lowering pollutants and unburned hydrocarbons in tailpipe exhaust. Butanol has 113 and 94 research and motor octane ratings, respectively, whereas ethanol has 111 and 92. Some of the benefits of butanol as a fuel have been recorded in the literature, such as a vapor pressure of 0.63 psi for pure butanol versus 2.25 psi for ethanol, and a heat of vaporization of (141.3 kcal) kg1 for butano versus (204.1 kcal) kg1 for ethanol. However, butanol's high boiling point (1188C) and reduced vapor pressure may make it difficult to start cold. It is more miscible with "gasoline" and "diesel fuel" than water [8]. Butanol is used in the chemical sector as well as a fuel. It helps to keep the air clean by lowering pollutants and unburned hydrocarbons in tailpipe exhaust [9].

MATERIALS AND METHODS

Isolation of Bacteria from Cow`s Feces:

Samples of Cow feces were obtained and serial dilutions made from the same sample, with the media containing (0.5gm KH2PO4 and 0.01gm FeSO4). 1 gm MnSO4.H2O, 0.15 gm CaCL2.2H2O, 5 gm yeast extract, 1 gm MgCL2.6H2O, 1.5 gm SO4 (NH4) 30 g glucose, 10 ml vitamin solution, 5 g Thiamine-HCL, 0.005g, Riboflavin0.005 gm Nicotinic acid, 0.0001gm B12, 0.005gm p-aminobenzoic , 0.005 gm lipoic acid and put in continous aeration incubator. The bacteria were then grown on nutrient agar plates [10]. Colonies were recovered and purified from 2 to 10 days of incubation at 37°C in a 250 ml shake flask containing 50 ml medium,later used this medium with bacteria and streaking on fresh nutrient agar, then inoculation from the plates to broth [11].

Cellulolytic bacteria identification:

Different types of bacteria were inoculated onto cellulose agar plates from L-agar plates (2.5g) of precipitated cellulose, (0.5g)peptone ,(0.2g)dipotassium-hydrogen phosphate , (0.2g) magnesium sulphate , (0.4g) potassium carbonate ,(0.02g) calcium chloride ,ferrous sulphate and sodium chloride ,and (15g) Oxoid ion agar per liter) and incubated at 37°C On this medium, only bacterial strains that can degrade cellulose thrive [10]. Several of cellulolytic bacterial strains will

be isolated and identified to the genus level using morphological and biochemical characteristics found by the kit [11 and 12].

Detection of Released Glucose Using Cellulose Saccharification and CMC

The saccharification of cellulose was determined using cellulose broth containing 0.5 percent cellulose (w/v) and all other ingredients contained in the cellulose agar medium except agar. Instead of cellulose, 0.5 percent CMC (w/v) was used to make CMC broth. Bacterial cultured sample (0.5 ml) Inoculate 50ml of new broth in 250ml flasks with cells grown to the same cell density in these broths. These cultures were cultured for 24 hours at 37°C with constant shaking before being centrifuged at 5000g for 10 minutes. The supernatants were boiled for 10 minutes with 5ml o-toluidine, and the OD635 was measure A standard calibration curve of glucose concentration (0-15 mmol/l) was used to determine the corresponding glucose concentration. [12].

Detection of Productive Butanol Concentrations

Samples were serially diluted from 10% to 50% and inoculated in a special growing medium to promote cellulosic bacteria for butanol production .To detect the products, the serial diluted tube with sample and media was incubated at 37^{o}C for 2 -10 days. The tubes were then ingected in GC to read the results after being treated with hexane [13].

Cellulase Production

Plates of cellulose agar (Carboxy methyl cellulose (0.5gm), Thermos agar (100ml), pH 7.0 adjusted before autoclaving at 1210C for 15 minutes) and was cultivated with a 24-hour-old bacterial culture [14]. The plates were covered with aqueous Congo red reagent Congo red stain 0.1 gm after 24 hours of incubation at 500C. The (Congo red stain was dissolved in distilled water to a volume of 100 ml). Cellulase producers were surrounded by yellow zone on red medium for 15 minutes and washed with NaCl 1M multiple times [15].

Detecting Glucose in paper wastes:

papers treated previously by 18% active alkali as NaOH was added into the heating glass tube and certain amount of fresh water was also added to make 4:1 bath ratio (liquid to solid ratio). Subsequently, the tube was kept in water bath, where water as a heating medium. Cooking of the material was performed at a temperature of about 100-120 °C for 60 min . After cooking, the tube was washed thoroughly with hot and afterwards with normal water to take away the black liquor from the tube until the pH of the filtrate reached to neutral level.

Then the 70g of paper yild was treated with a bacteria-produced enzyme (cellulase) *Serratia liquefaciens* and stored to detect the librated Glucose (3.2) that was used to make butanol. [11 and 15].

RESULTS AND DISCUSSION

The obtained samples of animal waste (cow feces) were processed and inoculated in a special growth medium that increased bacterial growth and butanol synthesis (Temp. 37°C and presence of (O2), as in the previous study [16] ,Which investigated the influence of component concentrations in a synthetic medium on Clostridium acetobutylicum fermentation of acetone and butanol, and discovered the impact of various key chemicals and anaerobic conditions on the product of butane, aceton, and methane, as well as bacterial activity. However, in this study, the obtained bacterium that was properly growth under

necessary aerobic circumstances and in particular medium conditions was *Serratia liquifaciens*, which was identified and diagnosed using a kit as shown in Figure (1). [17]

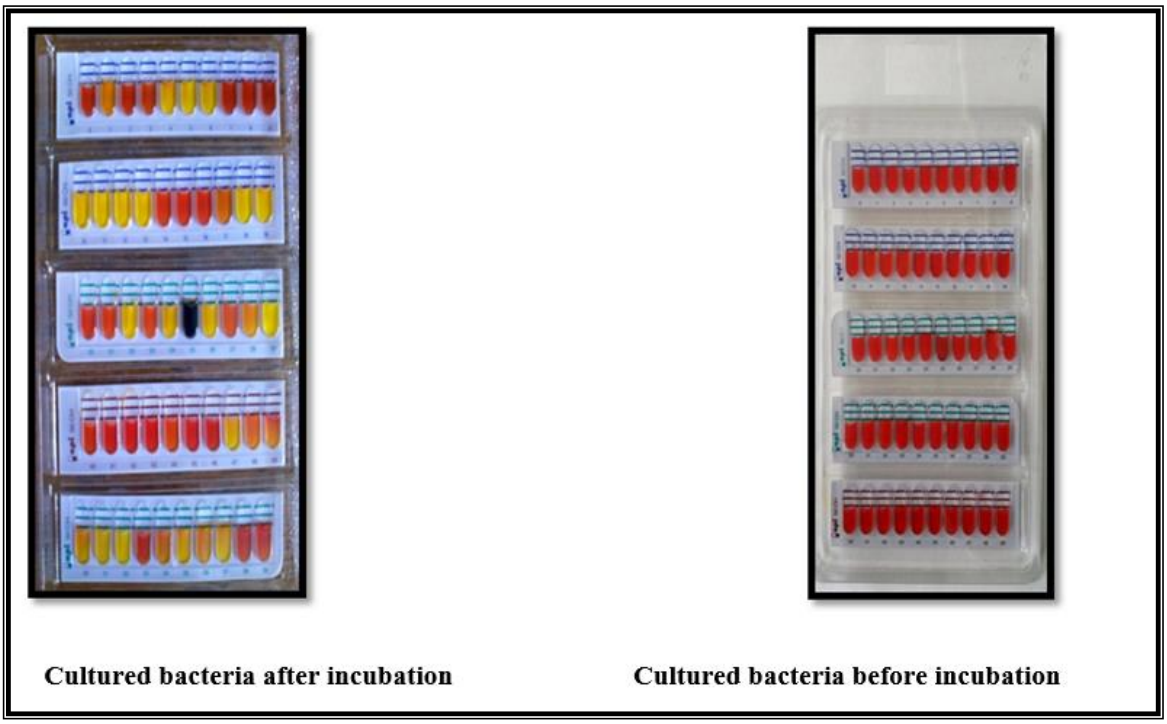

Figure (1): State the cultured bacteria in the kit before and after incubation

In samples relevant to the formation of butanol, each sample recorded OD at 640 and the concentration of librated Glucose from breakdown cellulose. as shown in Table (1)

Table (1): State the OD and concentration of Glucose in serial tubes.

No.	OD	Co. of Glucose
1	0.004	19 mg/l
2	0.0066	18.3 mg/l
3	0.013	22mg/l
4	0.014	23 mg/l
5	0.39	22mg/l
6	0.66	24mg/l

[18] studied the production of butanol In a batch fermentation with optimized inoculation ratio of 5 % C. acetobutylicum TSH1 and 0.5 % B. cereus TSH2, 11.0 g/L butanol and 18.1 g/L ABE were produced under microaerobic static condition. In contrast to the single culture of C. acetobutylicum TSH1, the symbiotic system became more aerotolerant and was able to produce 11.2 g/L butane. But according to our research the production of butanol reached to 164g/l.

[19] studied the production of butanol from agricultural and animal wastes and found 19.1 g/l of butanol produced under anaerobic conditions, in contrast to the simple aerobic conditions of this study.

Butanol production aerobically in this study and reached 164.6g/l and varied with time of culture; for example, it reached a maximum production within 10 days as show in figure (2) and figure (3).

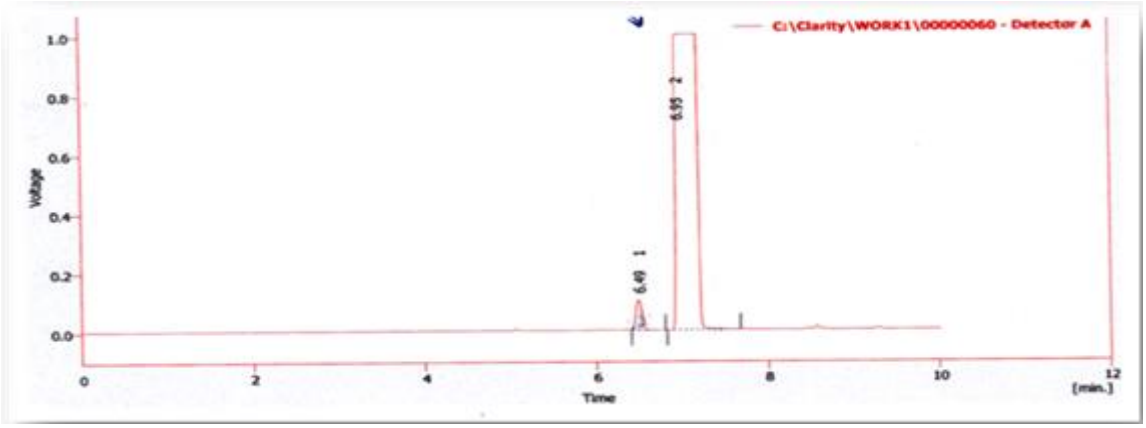

Figure (2) State the standard curve of butanol (control)

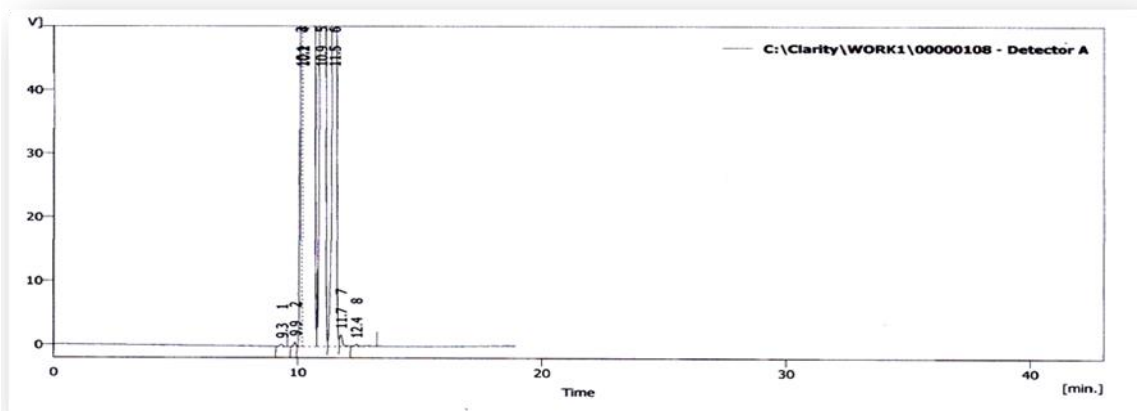

Figure 3.State the curve of butanol after 10 days incubation

The yield of microaerobic culture process was high during the 4 days of experiment and then decline slowly to stay stable to the last 10 day as shown in figure (4).

As shown in Figure (2) production was highly affected by initial population of bacteria on a medium containing 50 g/L glucose under "isolated microaerobic" conditions was accompanied by production of 164 g/L depending on initial population of Serratia liquifaciens strain Initiating the coculture with 1 mg/L of Serratia liquifaciens. [18] reported similar results for a culture with C. acetobutylicum. The profile of glucose consumption (Supplementary Materials) indicated that the outgrowing of Serratia sp. at high in the ratio of initial population (RIP) resulted in overconsumption of glucose by Serratia sp. And hihgh butanol production until reach to stable stage then decline in butanol product accompanied by the lower population of bacteria.

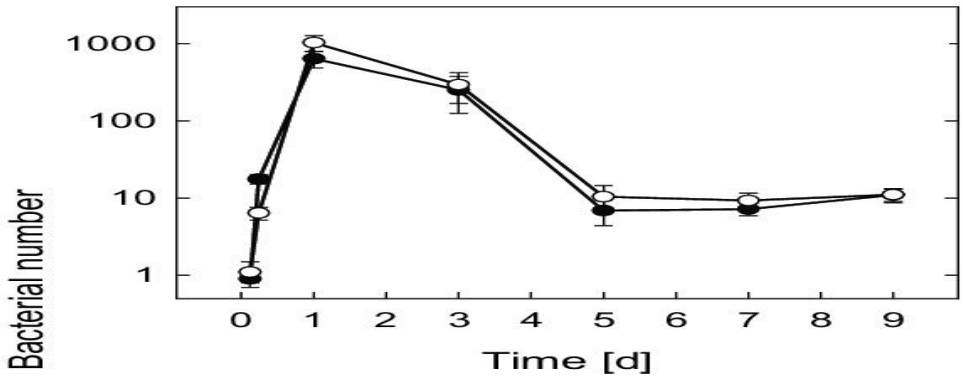

Figure (4) state the growth rate of *Serratia liquifaciens*

It`s found, there are no significant changes between the incubation times of 2days and 10 days.

As shown in Figure (5) [20 and 21].

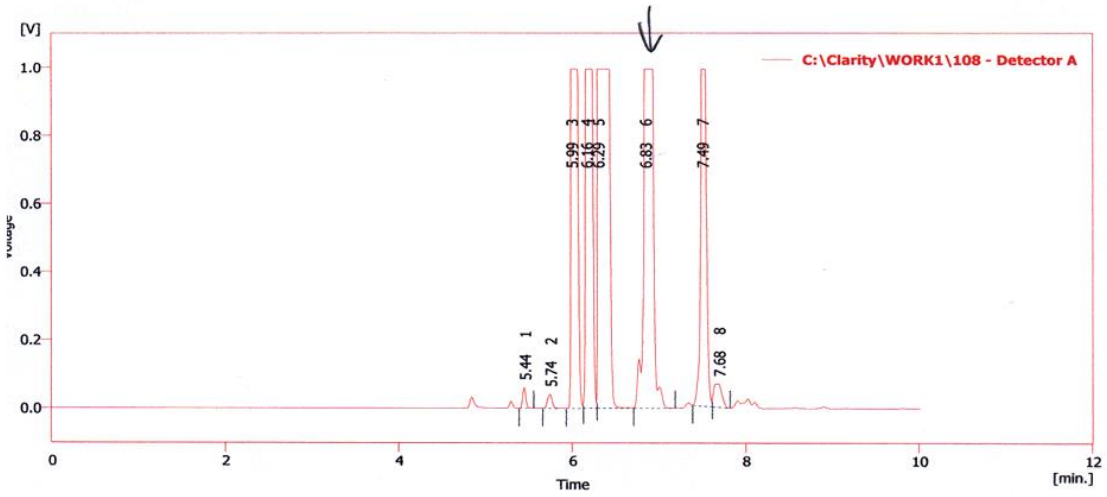

Figure (5) state the butanol level after 10 days' incubation

According to the paper wastes, the same bacteria were used to release cellulase in the degradation of 70 g of paper waste residue, with a level of butanol reaching 19.7g/l. as shown in figure (6).

This method used papers treated previously by active alkali treatment to obtain fine sample of paper used easly for glucose and then butanol production [22].

Figure (6) State the concentration of butanol product from paper waste residue.

Furthermore, the optimized results of using Serratia sp. strain Fas an auxiliary microorganism are better than other coculture processes. Improving the rate of the culture process production after optimizing the effective parameters could reveals the high potential of this culture method for using in the butanol production and other biochemical processes.

Conclusions

Serratia liquifaciens can be found in the digestive tracts of animals such as cows and can be forced to manufacture butanol aerobically. According to the anaerobic conditions that require hard effort and designed product for the products, this bacteria produced a large proportion of butanol under simple and available conditions reached to 164g/l by using animal feces as glucose source and, the yield of butanol by using paper wastes as glucose source was 19.7g/l. The optimum conditions for butanol production by Serratia liquifaciens were included culturing aerobically in the media consist of (0.5gm KH2PO4, 0.01gm FeSO4.7H2O,0.01gm MnSO4.H2O,0.15gm CaCL2.2H2O, 5gm yeast extract,1gm MgCL2.6H2O,1.5gm SO4(NH4)2, 30gm Glucose, 10 ml vitamin solution, 5gm Thiamine-HCL, 0.005gm Riboflavin,0.005 gm Nicotinic acid, 0.0001gm B12, 0.005gm p-aminobenzoic, 0.005 gm lipoic acid in 37^0C.

Recommendations

Investigate the genetics of butanol production by looking into a specific gene in bacteria. Molecular biology is used to identify microorganisms. Improving the efficiency of butanol production using genetic engineering.

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